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THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES

MAY, 1946

ORIGINAL ARTICLES

NUTRITIONAL MACROCYTIC ANEMIA, ESPECIALLY IN PREGNANCY

RESPONSE TO A SUBSTANCE IN LIVER OTHER THAN THAT EFFECTIVE IN PERNICIOUS ANEMIA*

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RECENTLY we have studied 4 patients with nutritional macrocytic anemia who showed no response to the parenteral administration of adequate doses of liver extracts regularly effective in Addisonian pernicious anemia. Three of these patients immediately thereafter responded to liver extracts given orally; the 4th, to a larger amount of the same preparation given parenterally. Since this indicated response to a substance other than the hematopoietic factor presumably common to all liver extracts effective in Addisonian pernicious anemia, it seemed desirable to present these cases and to review apparently similar cases, which so far have been reported only in the foreign literature.

One of us[8,14] in 1929 postulated that the hematopoietic factor effective in Addisonian pernicious anemia is normally formed by the interaction of a gastric (intrinsic) and a food (extrinsic) factor. It was demonstrated that in Addisonian pernicious anemia the intrinsic factor is usually absent. Evidence obtained later[9,45] suggested that in other types of macrocytic anemia also, the specific factor in liver extract is lacking as a result of absence of food (extrinsic) or of gastric (intrinsic) factor; of defective absorption of their reaction product from the gastro-intestinal tract; or of some combination of these pathogenic factors. Thus, the unitarian concept was set forth[10,11] that pernicious and related macrocytic anemias had in common the characteristic of responding to oral or parenteral administration of a single active principle in liver extract. The extract which was employed at that time was a fraction of liver soluble in 70% alcohol but precipitated when the concentration of the alcohol was raised to 95% by volume; that is, Fraction G of Cohn, Minot and their associates.[15] Consistent

* The expenses of this investigation were defrayed in part by the J. K. Lilly gift to the Harvard Medical School.

† Commonwealth Fund Fellow.

with this hypothesis appeared to be the regularity of response to parenteral administration of such relatively crude liver extracts observed in Addisonian pernicious anemia;[24,46] in the macrocytic anemia of the tropics[36,61] and of tropical[12,30,50] and non-tropical sprue;[41] and in macrocytic anemia associated with abnormalities of the gastro-intestinal tract,[2,49] dietary (extrinsic factor) deficiency,[27] pregnancy,[34,42] and fish tapeworm infestation.[6] However, since 1938 there have appeared in the foreign literature scattered reports of cases of macrocytic anemia which have responded poorly, or not at all, to the parenteral administration of the refined liver extracts that first became generally available at about that time. These patients have subsequently responded either to less refined liver extracts given parenterally or, failing in this, to liver or other preparations given orally.

Review of Literature. The clinical observations on such differences in therapeutic activity of liver extracts of various degrees of refinement were foreshadowed in 1937 by the results of experimental studies in monkeys, made by Wills, Clutterbuck and Evans.[60] These workers showed that in contrast to a relatively crude liver extract, Campolon,[26] a purified liver extract, for example Anahaemin,[16,53] was therapeutically ineffective. Napier and his associates[36] in 1938 reported that of 15 patients with tropical macrocytic anemia which they observed in India 3 failed to respond to parenterally administered Anahaemin, but subsequently responded to parenterally administered Campolon. Wills and Evans[61] in the same year reported 26 cases, also from India, in which Anahaemin was ineffective but in which there subsequently occurred a response to Campolon or to the oral administration of Marmite (autolyzed yeast). Later, Napier[35] reported that Anahaemin, if given in massive doses, would sometimes produce a response, although it was never as effective as Campolon. Trowell[51] reported that his patients in Uganda seemed to require more Anahaemin or more Campolon than did patients with pernicious anemia, and he regarded liver given by mouth as the treatment of choice. On the other hand, Foy and Kondi[24] and Fairley[23] found that nutritional macrocytic anemia in Macedonia responded equally well to Anahaemin and to cruder preparations for parenteral use, such as Campolon. Rodriguez-Molina[40] observed a patient with tropical macrocytic anemia in Puerto Rico who responded well to autolyzed yeast after showing no response to liver extract given intramuscularly.

In addition to the tropical macrocytic anemias, there have been cases of macrocytic anemia of pregnancy in the temperate zone, which have not responded satisfactorily to parenterally administered refined liver extract. Ungley[52] in 1938 published, without specific comment, the chart of a patient who failed to respond to a 10-day course of Anahaemin, but who subsequently showed a striking response to oral therapy with a combination of iron, Marmite, orange juice, liver and kidney. In 1942 Davidson, Davis and Innes[17] reported 16 cases of megaloblastic anemia of pregnancy, 10 of which were temporarily refractory to refined and crude liver extracts, such as Anahaemin and Campolon, administered parenterally for periods varying from 11 days to several months. In the same year Miller and Studdert,[31] in reporting 23 cases of pernicious anemia of pregnancy, stated that 1 patient responded to a diet rich in vitamin B after showing no response to "pure" liver extract given parenterally; and that 5 patients responded to raw liver, 2 of them after failing to respond to Anahaemin, given parenterally. Unfortunately individual cases were not described in detail. Fullerton[25] in 1943 reported 3 cases of macrocytic anemia of pregnancy "of interest because there was little or no improvement in the blood level after liver extracts given parenterally, but rapid regeneration occurred when this treatment was supplemented by the ingestion of whole liver." Scrutiny of the hematologic data, however, indicates that such a clear cut interpretation of the results is difficult, as the writer himself admits. It should be emphasized that in contrast to these investigators who claim to have found that parenterally administered refined liver extract was therapeutically inadequate in cases of pernicious anemia of pregnancy, there are others[1,29,34,42,44] who have found this treatment satisfactory in the majority of their cases. The literature on the subject of the anemias of pregnancy has re-

cently been ably reviewed by Callender[7] and by Elliott.[21]

Finally, there have been reported a few miscellaneous cases of nutritional macrocytic anemia in the temperate zone with atypical response to liver extract therapy. Nielsen[38] in 1941 stated that he had studied 4 cases in Denmark which did not respond to liver extract given parenterally or to stomach preparations given by mouth, but which did respond to orally administered liver extract. Analysis of these cases reveals that only 1 was adequately controlled. This patient responded to the oral administration of autolyzed yeast after showing no response to parenterally administered refined liver extract, Pylorin (desiccated hog stomach pylorus), lactoflavin and Ventriculin, N. N. R. Fullerton[25] mentions briefly 3 cases of macrocytic anemia associated with steatorrhea, which were refractory to extracts administered parenterally but responded rapidly when they were given whole liver without other alteration in the diet. Of particular interest is the report by Davidson, Davis and Innes[18] in 1943 of 6 cases of severe idiopathic macrocytic anemia with megaloblastic sternal marrow, all of which were refractory to liver extract given parenterally for periods of from 4 to 10 weeks, but eventually recovered. Orally administered liver was not tried in these patients, but recently Davis and Davidson[19,20] have demonstrated most convincingly in 5 instances that such refractory anemias respond at once to orally administered "proteolyzed liver." In retrospect there is also perhaps therapeutic significance in the report by Bomford and Rhoads[5] of "spontaneous remissions" in 6 cases of refractory anemia, in 3 of which (Cases 18, 19 and 21) these remissions occurred during treatment with orally administered liver or liver extract.

As a group, these cases of nutritional macrocytic anemia described by other authors seemingly failed to respond to refined or in some instances to crude liver extracts given parenterally, and thus differed from cases of Addisonian pernicious anemia. Unfortunately some of the reports lacked such pertinent hematologic data as reticulocyte counts, or such essential therapeutic controls as demonstration of failure to respond to parenterally administered liver extract immediately preceding response to an orally administered product. Moreover, therapy often included several agents given simultaneously, so that it is difficult to conclude which was effective. The present report of observations on 4 patients with macrocytic anemia supplements a preliminary communication[57] and appears clearly to demonstrate response to a hematopoietic substance other than that effective in Addisonian pernicious anemia, whether given by the oral or by the parenteral route.

Methods. On the basis of previous experience with pernicious anemia,[8,11] one or more successive 10-day periods of daily administration were considered adequate to establish the hematopoietic activity of a test preparation, chiefly by the absence or presence of a significant reticulocyte response.[8,32] The percentage of reticulocytes in 1000 red blood cells was determined daily from blood films vitally stained with brilliant cresyl blue and counterstained with Wright's stain. Complete blood counts, including hematocrit and corpuscular indices, were made on alternate days from 5 cc. of venous blood placed in a bottle containing 6 mg. of ammonium oxalate and 4 mg. of potassium oxalate crystals. During the periods of critical observation the patients were kept on diets containing no meat, fish, eggs, liver or hematopoietic substances other than those under test. The results of these observations are presented graphically in Figures 1 to 5.

Discussion. *Evidence that the basic nutritional deficiency differs from that in Addisonian pernicious anemia.* All 4 of our cases of nutritional macrocytic anemia, like others reported,[7,35,58] possessed in common certain characteristics which distinguish them from cases of Addisonian pernicious anemia. The most important features seemed to be a history of marked dietary inadequacy, especially with respect to meat, and the presence of free hydrochloric acid in the gastric contents. Additional distinguishing characteristics were absence of neural manifestations and

of atrophy of lingual papillæ; less macrocytosis, anisocytosis and poikilocytosis of the red blood cells than is usual in pernicious anemia; an unusually low percentage of reticulocytes; and a normal icterus index. Other observers have noted that the bone marrow in such cases is megaloblastic[20,35] and that maintenance liver extract therapy is not required.[18] Our patients have not been followed sufficiently long to permit definite conclusions, but the 3 patients who have taken no liver extract for over a year have suffered no relapses.

In Case 2, the presence of free hydrochloric acid in the gastric juice and the absence of response to daily therapy with 1 cc. of Reticulogen-Lilly and 4 U. S. P. units (injectable) of Liver Injection (Crude), U. S. P.-Wilson eliminated the possibility of typical Addisonian pernicious anemia (Fig. 2). In this case, too, the blood picture and the hemorrhagic phenomena were suggestive of aplastic (refractory) anemia; but here again there was a striking response to orally administered Liquid Extract of Liver, U. S. P.-Valentine, 1 U. S. P. unit (oral) daily.

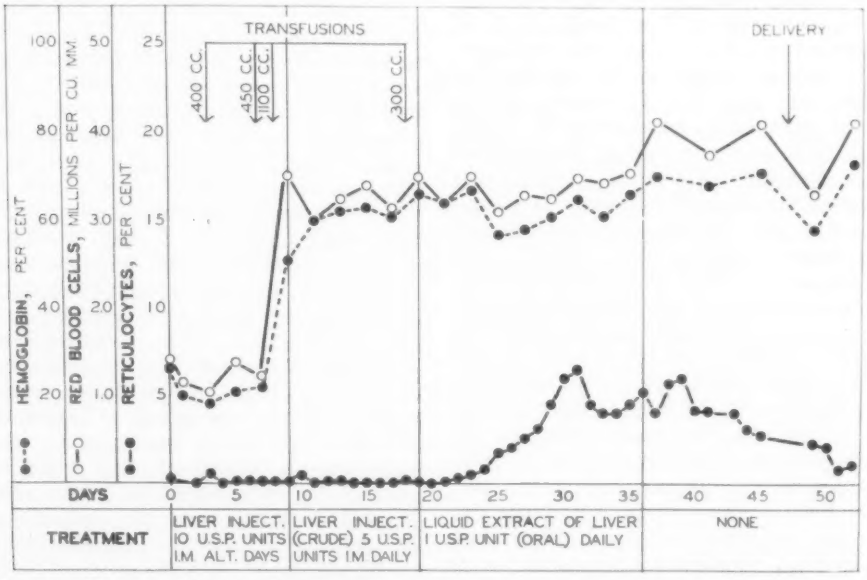

FIG. 1.—(Case 1.) Nutritional macrocytic anemia of pregnancy. Response to orally administered Liquid Extract of Liver, U. S. P.-Valentine after failure to respond to parenterally administered Liver Injection, U. S. P.-Lederle and Liver Injection (Crude), U. S. P.-Wilson.

Case 1 was fairly typical of pernicious anemia of pregnancy. When there was no response to several U. S. P. units (injectable) daily, first of Liver Injection, U. S. P.-Lederle[54] and then of Liver Injection (Crude), U. S. P.-Wilson,[55] the diagnosis of pernicious anemia was excluded, and aplastic (refractory) anemia seemed likely (Fig. 1). Subsequently, however, there was a prompt response to orally administered Liquid Extract of Liver, U. S. P.-Valentine,[37,39] 1 U. S. P. unit (oral) daily.

Because the daily injection of a single U. S. P. unit is sufficient to produce a striking hematologic and clinical remission in uncomplicated Addisonian pernicious anemia,[54] the therapeutic failure of these potent liver extracts when given parenterally clearly indicates that *the nutritional deficiency in these patients did not consist in a lack of that active principle of liver extract which is effective in Addisonian pernicious anemia.*

According to the hypothesis of Strauss

and Castle,[45] deficiency of the active principle of liver extract which is effective in Addisonian pernicious anemia is the result of lack of either the so-called food (extrinsic) factor or the gastric (intrinsic) factor, or is due to defective absorption of their reaction product from the intestinal tract. Therefore, because the macrocytic anemia in our patients failed to respond to the parenteral administration of liver extracts effective in Addisonian pernicious anemia, it is clear that deficiency of extrinsic or intrinsic factors or defective absorption of their reaction product is not the basis of this report differs from that in Addisonian pernicious anemia, what is the explanation of the hematopoietic activity exhibited by the orally administered Liquid Extract of Liver, U. S. P.-Valentine? Two obvious possibilities present themselves: (1) that the administration of the material by the oral route is necessary for the elaboration of some active principle different from that effective in Addisonian pernicious anemia; or (2) that an effective amount of the different active principle is already present in the prepara-

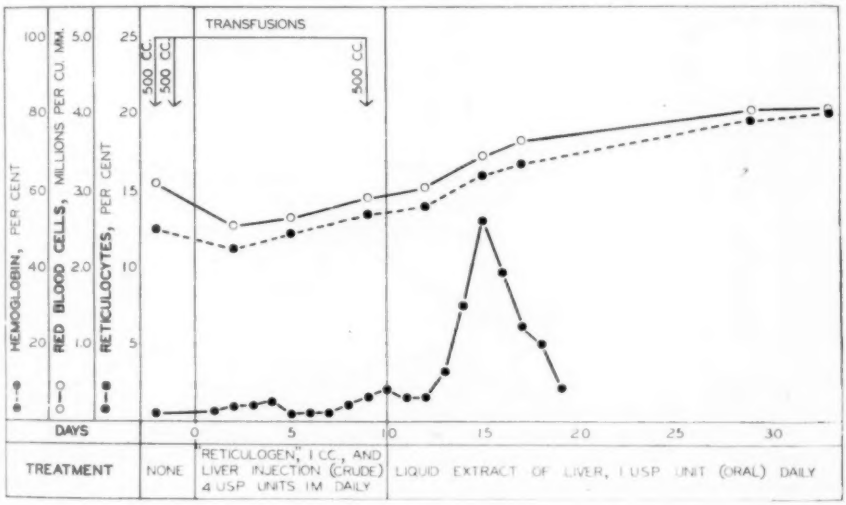

FIG. 2.—(Case 2.) Nutritional macrocytic anemia. Response to orally administered Liquid Extract of Liver, U. S. P.-Valentine after failure to respond to parenterally administered Reticulogen-Lilly and Liver Injection (Crude), U. S. P.-Wilson.

its cause. Consequently the unitarian theory of the etiology of macrocytic anemias of nutritional deficiency, which has been advanced by Strauss and Castle, does not apply to the patients presented in this report. This conclusion was first clearly reached in 1938 by Napier and his associates[35,36] and by Wills and Evans[61] for certain macrocytic anemias occurring in the tropics, which failed to respond to refined liver extracts given parenterally.

Evidence concerning the nature of the hematopoietic agent. If, then, the nutritional deficiency of the 4 patients forming the basis of this report differs from that in Addisonian pernicious anemia, what is the explanation of the hematopoietic activity exhibited by the orally administered Liquid Extract of Liver, U. S. P.-Valentine? Two obvious possibilities present themselves: (1) that the administration of the material by the oral route is necessary for the elaboration of some active principle different from that effective in Addisonian pernicious anemia; or (2) that an effective amount of the different active principle is already present in the preparations administered orally but not in those administered parenterally.

The observations in Case 3 were accordingly designed to discover a liver extract which was orally effective in nutritional macrocytic anemia of the type under study and which could also be given parenterally. Based on previous experience in pernicious and related macrocytic anemias,[12,46,47,48] a "special liver preparation" was now employed. This contained in 5 cc. as a suspension-solution, at pH 6.8, 1.25 gm. of powdered Liver Extract-Lilly, N. N. R.[37] Case 3 resembled Case 1

in clinical and hematologic aspects except that delivery took place on the day that the observations were begun. In a first period of 8 days the patient was given daily injections of 5 cc. of the special liver preparation without detectable effect* (Fig. 3). Owing to the serious condition of the patient it was then considered advisable to discontinue parenteral therapy. Beginning on the 9th day, therefore, the patient was given daily, by oral administration, 51 cc. of the same special liver preparation, containing 12.75 gm. of

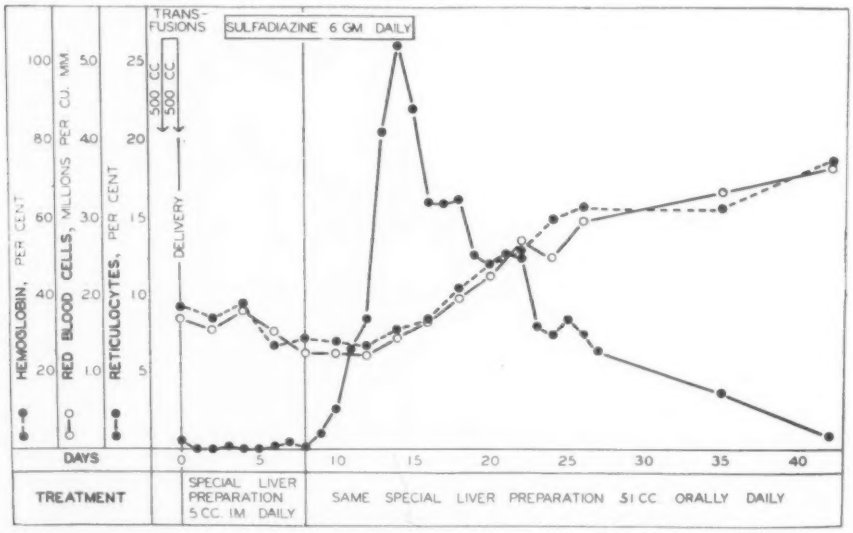

FIG. 3.—(Case 3.) Nutritional macrocytic anemia of pregnancy. Response to orally administered special liver preparation (suspension-solution of Liver Extract-Lilly, N. N. R.) after failure to respond to the *same* preparation given intramuscularly.

ceivably delay or failure of response to material administered during the first period could have been caused by an infection responsible for the fever which was present from the 4th to the 16th day. However, because no locus of infection was demonstrable, the fever was probably due to the anemia and in any case was present during as well as before the period of active hematopoietic response.

The observations in Case 3 indicate that the special liver preparation was active on oral administration. Thus, the powdered Liver Extract-Lilly, N. N. R.,[37] or the equivalent of 1 U. S. P. unit (oral). Under this therapy the reticulocytes increased rapidly and on the 7th day reached a peak value of 26.2%. It is considered unlikely that this rise was due to a delayed response to the material administered parenterally during the first period because in uncomplicated pernicious anemia at least, large reticulocyte responses almost never begin as late as the 10th day,[32] especially with parenteral therapy. Consuspension-solution of Liver Extract-Lilly, N. N. R.,[37] containing material soluble in 70% but insoluble in 95% alcohol by volume,[15] like the simpler aqueous extract,[39] Liquid Extract of Liver, U. S. P.-Valentine[37] (Cases 1 and 2), was shown to supply a hematopoietic factor different from that effective in Addisonian pernicious anemia. The fact that this identical special liver preparation was active on oral administration but not on injection suggested the first possibility referred to above; namely,

* Tests of the potency of this preparation in patients with Addisonian pernicious anemia showed that 2 cc., when injected daily, were equivalent to about 1 U. S. P. unit (injectable) of certain official U. S. P. preparations.

that in order to become active hematopoietically, the liver extract requires access to the gastro-intestinal tract, perhaps there to be acted on by some secretion in a manner analogous to the extrinsic-intrinsic factor mechanism in Addisonian pernicious anemia. Consistent with this idea was the demonstrated presence of hydrochloric acid in the gastric contents of this patient as well as of the other patients reported here. The second possibility, however, offered the simpler explanation: that a greater amount of the unknown active principle (other than that effective in pernicious anemia) was given when the material was administered orally than when it was injected. Thus, Case 3 received 5 cc. of the special liver preparation daily by intramuscular injection without effect, but thereafter received daily 51 cc. —10 times as much—of the same material by mouth with immediate and striking hematopoietic results (Fig. 3).

Case 4 was therefore studied in an attempt to ascertain whether the oral route of administration or the amount of the preparation given was of prime importance. This patient had nutritional macrocytic anemia with a history of prolonged dietary inadequacy. Free hydrochloric acid was present in the gastric contents. Addisonian pernicious anemia was excluded by the absence of hematopoietic response, in an initial 10-day period, to the daily intramuscular injection of 2 cc. of the special liver preparation, the suspension-solution of Liver Extract-Lilly, N. N. R., estimated to be equivalent in hematopoietic activity to about 1 U. S. P. unit (injectable) (Fig. 4). In a third period the patient received daily intravenous injections of 20 cc.—10 times as much—of the supernatant (98.5% by volume) of the special liver preparation and showed a prompt rise in reticulocytes to a peak of 25.1% on the 6th day. Since the only essential difference between the two therapeutic periods was the *amount* of liver extract given, it can be concluded that the response was due to the administration of a greater and thus adequate amount of some effective hematopoietic substance

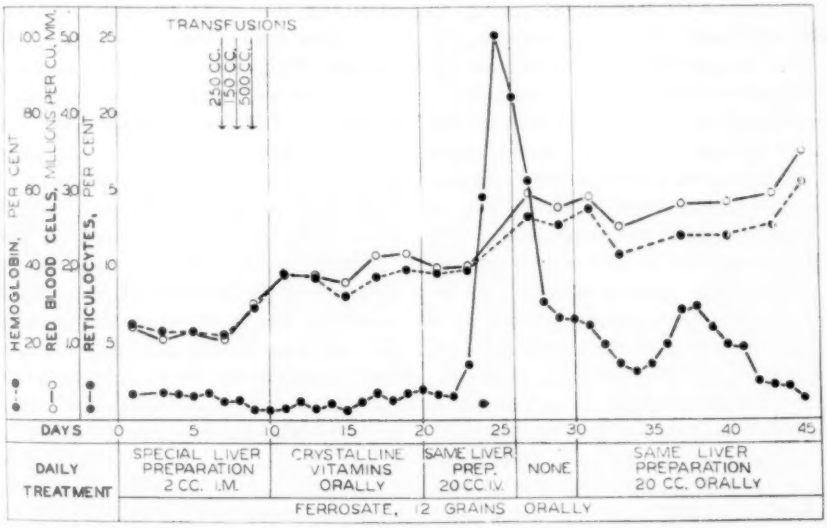

FIG. 4.—(Case 4.) Nutritional macrocytic anemia. Failure of response to various pure components of the vitamin B complex and accessory nutritional substances, including biotin, *L. casei* factor and pyridoxal. Response to daily parenteral administration of 20 cc. of special liver preparation (solution of Liver Extract-Lilly, N. N. R.) after failure to respond to the daily parenteral administration of 2 cc. of the *same* preparation.

other than the antipernicious anemia factor. Consequently, it appears that administration by the oral route is not necessary for effective response.

As indicated by the details of the report of Case 4, certain special features need discussion. The day preceding the reticulocyte peak in the third period the patient developed bronchopneumonia, but despite the presence of infection the reticulocyte response was striking. Since there was no evidence of infection in the first period, absence of response at that time cannot be attributed to this adverse factor. The liver extract was given intravenously in the third period rather than intramuscularly as in the first period because the daily intramuscular injection of 20 cc. of liver extract would have been too painful to be tolerated by the patient. No evidence exists of therapeutic failure in pernicious anemia because of lack of absorption of liver extracts given intramuscularly, and persistence of such an effect over several days is certainly unlikely in any patient.

An oral test made subsequently in Case 4 with 20 cc. daily of the same material that had previously been given intravenously (Fig. 4) resulted in a second reticulocyte rise to a peak of 7.4% on the 9th day. Such suggestive, though not conclusive, evidence of comparable potency of this preparation on oral administration presents a complete contrast to our experience in Addisonian pernicious anemia, in which its activity when orally administered has been found to be only about 1/60 of that when injected.[48] This observation suggests that the absorptive capacity of the alimentary tract in this patient was not significantly decreased, as it often is in patients with pernicious anemia, and constitutes evidence of an additional physiologic difference from pernicious anemia.

The chemical nature of the hematopoietic factor or factors effective in these patients is still obscure. Wills and Evans[61] and Davis and Davidson[20] have hypothesized that both the principle effective in Addisonian pernicious anemia and an additional substance are required. An opportunity has not yet presented itself for determining whether the most refined liver extracts, if given in sufficient amounts, would still be ineffective and consequently would be judged as completely lacking in the additional substance. However, because in our hands Fraction G of Cohn, Minot and their associates[15] in the form of the special liver preparation was active both orally and parenterally, it is not necessary to suppose that the special properties of the so-called "proteolyzed liver" of Davis and Davidson[20] are essential. This, of course, does not deny that relatively unrefined preparations of liver, including proteolyzed liver, may actually contain more of the unknown active principle than do more refined preparations. Moreover, it is not possible to be certain that the basic deficiency in the cases reported by Davis and Davidson is identical with that in each of the cases which we have described.

The fact that the diets of our patients were particularly deficient in animal protein does not imply that the dietary deficiency was necessarily due to protein itself, as Bethell[3,4] has postulated for the macrocytic anemias of pregnancy. Indeed, the responses in Cases 3 and 4 following administration of a liver extract which is essentially free of protein[15] clearly indicate that this effect could not have been due to protein as such. Nevertheless, requirement for a substance associated with animal protein, such as a member of the vitamin B complex, seems possible, as was suggested by Wills for her cases of tropical macrocytic anemia[59,61] and for a similar experimentally produced nutritional macrocytic anemia in monkeys.[60] Very recently the ineffectiveness of additional identified components of the vitamin B complex as extrinsic factor or as "liver factor" in Addisonian pernicious anemia has been demonstrated.[13] Case 4 likewise gave no response, during the second 10-day period, to the daily oral administration of large amounts of a mixture of the crystalline B vitamins and certain other accessory factors listed in the case sum-

mary, such as biotin, *L. casei* factor* and pyridoxal.[41,43]

Reconsideration of observations on macrocytic anemias ascribed to deficiency of so-called food (extrinsic) factor. Reports have appeared in the literature of patients with macrocytic anemia who have exhibited hematopoietic responses to autolyzed yeast,[12,56,58] dried yeast,[22,62] beef muscle,[12,33,47] or meat, eggs and milk.[27] Castle and his associates,[9–12] Groen and Snapper,[27] Kern,[28] Moore, Vilter, Minnich and Spies[33] and others have attributed such effects to an interaction between the food (extrinsic) factor in these various substances and effective amounts of gastric (intrinsic) factor co-existing with the hydrochloric acid usually present in the gastric contents of these patients. However, if such patients, like those in the present report, were actually incapable of response to usual doses of refined liver extract administered parenterally, the hematopoietic effects should not have been attributed to the oral administration of the food (extrinsic) factor, but rather to some other property of the materials. The 56 patients with macrocytic anemia associated with deficiency of the vitamin B complex, reported by Moore and his associates,[33] "showed prompt therapeutic response to the parenteral injection of highly purified liver extracts." Consequently, the responses to beef muscle, also demonstrated, may be considered as due to its content of extrinsic factor. As no tests with highly refined liver extracts were made in the cases of sprue studied by Castle, Rhoads, Lawson and Payne,[12] this causes some uncertainty with respect to their interpretation of the basis of effectiveness of beef muscle and milk and particularly of autolyzed yeast, which were hematopoietically active, respectively, in 8 and in 7 of 18 cases in which they were tried. However, unlike the patients in the present report, 8 of these 18 patients responded subsequently to 2 cc. amounts daily of parenterally administered liver extract (a solution of Liver Extract-Lilly, N. N. R.), as did 10 other patients. Except that the insoluble material had been removed, this liver extract was identical with that given as the special liver preparation without effect in 2 cc. and in 5 cc. amounts daily to Cases 4 and 3, respectively (see Figs. 4 and 3).

The interpretation of the observations of Strauss and Castle[45] on the etiology of the macrocytic anemias of pregnancy also needs critical re-examination. Case 5 of their report, as well as the first of the 2 additional cases synoptically described, demonstrates that normal human gastric juice was essential for the development of a significant hematopoietic response to the daily administration of 300 gm. of beef muscle or of 16 gm. of autolyzed yeast (Vegex), respectively. By contrast, in their Case 6 only insignificant reticulocyte responses occurred although beef muscle was given daily with hydrochloric acid and then, during 2 subsequent periods, with normal human gastric juice (Fig. 5). However, when 12.75 gm., or 1 U. S. P. unit (oral), of Liver Extract No. 343, N. N. R., (now called Liver Extract-Lilly, N. N. R.)[37] were given daily, a reticulocyte response of 46% and rapid hematologic and clinical improvement resulted. This observation suggests that the basic deficiency in Case 6 was not analogous to that in Addisonian pernicious anemia, but rather was like that in the cases reported in the present communication. If this is so, then 300 gm. of beef muscle, although containing adequate food (extrinsic) factor,[9,11] are *not* a good source of the unknown active in-

* Subsequent to the submission of this manuscript for publication, both oral and parenteral administration of distinctly larger amounts of *L. casei* factor have been shown to be hematopoietically and clinically effective in nutritional macrocytic anemias, including Addisonian pernicious anemia, sprue anp pernicious anemia of pregnancy. Whether patients similar to those reported here were included is not apparent from the published articles: DARBY, W. J., and JONES, E.: Proc. Soc. Exp. Biol. and Med., 60, 259, 1945; MOORE, C. V., BIERBAUM, O. S., WELCH, A. D., and WRIGHT, L. D.: J. Lab. and Clin. Med., **30**, 1056, 1945; SPIES, T. D., VILTER, C. F., KOCH, M. B., and CALDWELL, M. H.: South. Med. J., **38**, 707, 1945; VILTER, C. F., SPIES, T. D., and KOCH, M. B.: South. Med. J., **38**, 781, 1945.

gredient present in Liver Extract-Lilly, N. N. R. It is thus probable that whenever, in the past, beef muscle has been administered orally with benefit in cases of macrocytic anemia,[12,33,47] its activity has been due, as hitherto assumed, to its content of so-called food (extrinsic) factor. This conclusion is substantiated by the uniformity of response of their patients to beef muscle and gastric juice as well as to highly refined liver extract recently observed by Moore and his associates.[33] Thus, it is clear that within the group of pernicious anemia when administered parenterally, produced no response. It therefore seems appropriate and convenient to designate the effective principle—which is as yet unidentified and which is apparently present in liver, proteolyzed liver, certain liver extracts and autolyzed yeast—as "Wills' factor," and to describe these particular macrocytic anemias as due to deficiency of that factor. Perhaps it would also be well to include under the term nutritional macrocytic anemia instances of macrocytic anemia due both to direct

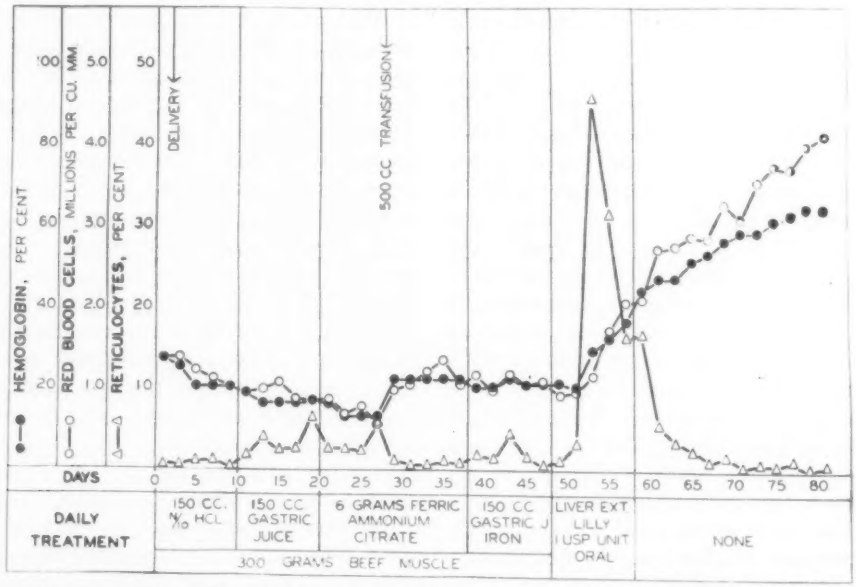
HEMOGLOBIN, PER CENT
RED BLOOD CELLS, MILLIONS PER CU MM
RETICULOCYTES, PER CENT
DELIVERY
500 CC TRANSFUSION
DAYS
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80
DAILY TREATMENT
150 CC. % HCL
150 CC GASTRIC JUICE
6 GRAMS FERRIC AMMONIUM CITRATE
150 CC GASTRIC J
IRON
LIVER EXT. LILLY 1 USP UNIT ORAL
NONE
300 GRAMS BEEF MUSCLE

FIG. 5.—(Case 6 of Strauss and Castle.[47]) Nutritional macrocytic anemia of pregnancy. Response to the oral administration of Liver Extract-Lilly, N. N. R., after failure to respond to the oral administration of beef muscle (extrinsic factor) and gastric juice (intrinsic factor).

cases of nutritional macrocytic anemia of pregnancy reported by Strauss and Castle, two therapeutically discrete varieties are now descernible.

Recognition and treatment of various types of nutritional macrocytic anemias. Dr. Lucy Wills deserves the credit for priority in demonstrating the effectiveness of autolyzed yeast in tropical macrocytic anemia of pregnancy[58] and for showing subsequently that in analogous anemias in monkeys,[60] certain refined liver extracts, although highly effective in Addisonian and to conditioned nutritional deficiencies. This generic use of the term would include Addisonian pernicious anemia and the macrocytic anemias of pregnancy, sprue, fish tapeworm infestation, and so forth, as well as the anemias more obviously associated with dietary deficiencies presumably including Wills' factor and the so-called extrinsic factor.

The clinical criteria for the selection of cases of nutritional macrocytic anemia due to deficiency of Wills' factor have been summarized above. The clinical distinc-

tion from Addisonian pernicious anemia is clear. However, except by means of testing the response to refined liver extract, it remains to be seen whether a sharp distinction can be drawn between instances of deficiency of Wills' factor and instances of extrinsic factor deficiency. The expectation, considering the tendency to the development of multiple deficiencies in human malnutrition, is in favor of combinations of the two deficiencies.

It is possible that in addition to patients who respond to parenterally administered refined liver extracts, and to others who respond only to parenterally administered crude liver extracts, there is a third group who will respond only to still cruder, and thus necessarily orally administered, extracts of liver or to autolyzed yeast. The existence of this third group was suggested by Napier and his associates[36] and can be inferred from such reports as those of Trowell,[51] Miller and Studdert,[31] Fullerton,[25] Nielsen[38] and Davis and Davidson.[19,20] Provisionally we have assumed that our 4 cases were alike in presenting the same nutritional deficiency. Case 4 responded to the special liver preparation when given intravenously in much larger amounts than could conveniently be given by intramuscular injection. Therefore the tentative conclusion can be drawn that the failure of the other 3 cases to respond to parenterally administered preparations was simply that these preparations, at least in the amounts given, did not contain enough Wills' factor to be effective. It is evident that the amount of Wills' factor does not parallel the U. S. P. unitage of these preparations, that is, their antianemic potency in Addisonian pernicious anemia. The fact that the principles effective in pernicious anemia and Wills' factor are apparently both present in certain liver extracts, and probably in varying proportions in different preparations, suggests an explanation of the conflicting experiences of others[23,24,36,61] with parenterally administered refined liver extracts in tropical macrocytic anemias.

It may be of practical therapeutic importance to keep in mind, in regard to patients with macrocytic anemia, that a failure to respond completely or partially to the parenteral administration of the usual dosage of a potent refined or crude liver extract should be followed by a trial of orally administered liver extract. For this purpose Liquid Extract of Liver, U. S. P.-Valentine and Liver Extract-Lilly, N. N. R., were found to be effective in the present observations, and no doubt similar products prepared by other pharmaceutical concerns would be equally so. If the patient is unable to tolerate oral medication, large amounts of an appropriate solution of Liver Extract-Lilly, N. N. R.,[48] given intravenously, as in Case 4, may be effective. It is possible, as is suggested by the experience of other investigators,[18,20,26] as well as by our observations on Case 2, that oral preparations may be of value in the temperate zone not only in certain patients with macrocytic anemias of pregnancy but also in occasional patients among those usually classified as having "aplastic," "achrestic," "refractory," or "atypical pernicious" anemia. However, at least in this country, anemias due to deficiency of Wills' factor probably present rare exceptions to the principles which have been established with respect to the etiology and treatment of pernicious and related macrocytic anemias, particularly the clearly demonstrated advantage and convenience of parenterally administered refined liver extracts of known effectiveness in Addisonian pernicious anemia.

Case Reports. CASE 1. D. D., a 29 year old housewife, 7½ months pregnant, was admitted to the Second Medical Service of Boston City Hospital on September 13, 1943. Ten months before admission she had a sore throat, cold and cough lasting 3 months, associated with anorexia, malaise and loss of 40 pounds in weight. Six months before admission she developed increasing weakness, dizziness, easy fatigability and dyspnea on exertion. As a result of associated anorexia her diet had been very inadequate. In the last few weeks she had noticed slight

afternoon fever, night sweats, sore tongue and difficulty in swallowing. She had had no paresthesias at any time.

Past History. The patient had had 7 previous pregnancies resulting in 6 living children, the 4th of whom was hospitalized for the first 6 weeks of life because of jaundice. She had not been anemic during any of these pregnancies. In the year preceding her present illness she had had most of her teeth extracted, but had not obtained dentures.

Physical Examination. Temperature 99.2° F.; pulse 72; respirations 22; blood pressure 115/65. The patient was thin and pale. No icterus, purpura, or adenopathy was present. The tongue was dry and heavily coated, but showed no papillary atrophy. There was complete upper edentia and partial lower edentia, with some pyorrhea. The heart was normal except for a systolic murmur, heard best in the pulmonic area. There was abdominal enlargement consistent with a 7 to 8 months' pregnancy; the liver and spleen were not palpable. Vibratory sense was intact; reflexes were normal; there was no edema.

Laboratory Findings. The blood on admission showed: hemoglobin 26% (4.1 gm. %); red blood cells 1,420,000 per cmm.; hematocrit 13.0%; M. C. V. 91.5 cμ.; M. C. H. C. 31.2%; M. C. H. 28.0 micro-micrograms; reticulocytes 0.4%; white blood cells 6400 per cmm., with a normal differential count. The stained smear showed slight anisocytosis, but no oval macrocytes, and a decrease in the number of platelets. The osmotic fragility of the erythrocytes was normal. The patient's blood was group O, Rh negative. Icterus index was 7½ units. Blood Hinton was negative. Blood non-protein nitrogen was 27 mg. %. The urine was negative except for Ehrlich's aldehyde reaction for urobilinogen, which was positive to a dilution of 1:128. Gastric analysis showed 1 cc. of N/10 free hydrochloric acid per 100 cc. of gastric juice in the fasting specimen and 2 cc. after histamine. The electrocardiogram was normal.

Course. The patient was considered to have pernicious anemia of pregnancy and was accordingly given intramuscularly, every other day over a period of 9 days, 1 cc. of Liver Injection, U. S. P.-Lederle* (Refined Solution Liver Extract Parenteral), containing 10 U. S. P. units (injectable) per cc. (Fig. 1). On the 4th day the patient was transfused with 400 cc. of whole blood. Because she showed no hematologic response, on the 7th and 8th days she was transfused with 450 cc. of whole blood and with the cells from 1100 cc. of blood, respectively. This brought the red blood cell count up to 3,500,000. During a second 10-day period the patient was given daily by intramuscular injection 2½ cc. of Liver Injection (Crude), U. S. P.-Wilson, containing 2 U. S. P. units (injectable) per cc. Again there was no increase in the reticulocytes or in the red blood cells. Beginning on the 20th day, for a third period of 17 days, the patient was given orally Liquid Extract of Liver, U. S. P.-Valentine,† 15 cc. 3 times daily, or 1 U. S. P. unit (oral). On the 6th day of this therapy the reticulocytes began to rise and reached a peak of 6.5% on the 13th day. On the 19th day the red blood cell count had reached 4,100,000. Coincident with the hematologic response there was marked improvement in the patient's appetite and in her general condition. Gastric analysis on the 13th day of therapy with orally administered liver extract showed an increase in acidity to 55 cc. of N/10 free hydrochloric acid per 100 cc. of gastric juice after histamine. On the 48th day after admission the patient gave birth to an Rh positive infant weighing 5 pounds and 6 ounces. This baby developed a mild hemolytic anemia of the newborn from which it recovered after several transfusions. The mother was discharged on November 17, 2 months after admission, in good health, and has remained so without further liver therapy. Blood studies 8 months after entry showed a hemoglobin of 94% and a red blood cell count of 4,760,000 per cmm. The platelets were abundant in the stained smear.

CASE 2. FIRST ADMISSION. A. M., a 35 year old housewife, was first seen on the Second Medical Service of the Boston City Hospital on August 5, 1942. In the 9 months before admission she had developed progressive fatigue, anorexia, pallor and dyspnea on exertion. Her dietary intake during this time had been relatively inadequate and as a result she had lost 50 pounds in weight. She had had one episode of sore tongue and mouth 3 months before admission. More re-

* Kindly supplied by Dr. Guy W. Clark of Lederle Laboratories, Inc.

† Kindly supplied by Mr. C. Braxton Valentine of Valentine's Meat-Juice Company.

cently she had had occasional vomiting, constipation, a burning sensation in her throat and a sense of numbness in her toes.

Physical Examination. Temperature 100° F.; pulse 120; respirations 35; and blood pressure 65/35. The patient was well nourished, but was very pale and appeared critically ill. Moderate dyspnea was present at rest. Both fundi showed numerous linear retinal hemorrhages. A small amount of pus was draining from the right auditory canal. The tongue was normal. The heart was slightly enlarged to the left, with a systolic murmur heard best in the pulmonic area. The liver was felt 3 finger-breadths below the costal margin, and was smooth, firm and non-tender. There was no splenomegaly, adenopathy, purpura, or icterus. Vibratory sense was normal; deep reflexes were active.

Laboratory Findings. Blood examination on admission showed: hemoglobin 12% (1.9 gm. %); red blood cells 500,000 per cmm.; hematocrit 5.1%; M. C. V. 102.0 $c\mu$.; M. C. H. C. 36.7%; M. C. H. 37.4 micro-micrograms; reticulocytes 0.6%; white blood cells 3400 per cmm., with a normal differential count. The stained smear showed moderate variation in size and shape of the erythrocytes, with polychromatophilia and Cabot rings but no oval macrocytes; multilobed neutrophils were common. The platelet count was 80,000 per cmm. The icterus index was 7 units. Blood non-protein nitrogen was 30 mg. %. The urine showed a maximum specific gravity of 1.023; initial specimens were loaded with white cells and contained albumin. Gastric analysis showed no free hydrochloric acid in the fasting specimen, but 10 cc. of N/10 free hydrochloric acid per 100 cc. of gastric juice were present after histamine.

Course. For the first 10 days the patient ran a fever ranging from 100° to 103° F., which was thought to be due to a urinary tract infection and which subsided on sulfadiazine therapy. During this first 10-day period the patient received one intramuscular injection of 5 cc. of Reticulogen-Lilly* and daily injections of 2 cc. of Liver Injection (Crude), U. S. P.-Armour,† containing 1 U. S. P. unit (injectable) per cc. There was no significant reticulocyte response during this time. However, 10 blood transfusions brought the red cell count to 3,010,000 per cmm. The patient was discharged improved on August 28, 23 days after admission, with a final diagnosis of aplastic (refractory) anemia.

SECOND ADMISSION. Sixteen months later, on December 22, 1943, the patient was readmitted. Until 2 months before entry, she had been seen monthly in the Out Patient Department, where she received an intramuscular injection of 1 cc. of Liver Injection, U. S. P.-Lilly (Solution Liver Extract Purified), containing 15 U. S. P. units (injectable) per cc. On February 10, 1943 her red cell count was 4,790,000 per cmm., and on October 20 it was 3,200,000. About once in 3 months since discharge she had had mild epistaxes from her right nostril.

Physical Examination. Physical findings were essentially the same as on the previous admission except that the patient did not seem so ill and there were no retinal hemorrhages.

Course. On admission blood examination showed: hemoglobin 35% (5.5 gm. %); red blood cells 1,330,000 per cmm.; hematocrit 15.8%; M. C. V. 118.8 $c\mu$.; M. C. H. C. 34.6%; M. C. H. 41.0 micro-micrograms; reticulocytes 0.7%; white blood cells 4900 per cmm., with a normal differential count. During the 22 days of her hospital stay she received transfusions totaling 2000 cc. of whole blood and 1000 cc. of red cells from which the plasma had been removed. She was given no liver extract because she was thought to have aplastic anemia. On discharge on January 13, 1944, her hemoglobin was 91%, and she felt moderately improved.

THIRD ADMISSION. On March 20, 1944 the patient was readmitted. She had led a bed and chair existence in the 2 months intervening. From February 25 to March 1 she had been on a surgical service where she had a fistula-in-ano excised under spinal anesthesia. She had continued to have weakness, dyspnea, general asthenia and severe anorexia. She had experienced mild nosebleeds, but on the day before admission she had a severe one which was finally stopped after 6 hours by a nasal pack inserted by her physician.

Physical Examination. The patient had marked pallor. There was a nasal pack in the right nostril. A few small purpuric spots were present on her legs. Otherwise the find-

* Kindly supplied by Mr. George B. Walden, of The Lilly Research Laboratories.
† Kindly supplied by Dr. John H. Glynn of Armour Laboratories.

ings were unchanged from the previous admission.

Course. The patient had a fever rising as high as 103° F. for the first 5 days, during which time she continued to have severe epistaxes. Sulfapyrazine therapy resulted in subsidence of the fever and disappearance of pus cells from the urine. Blood examination on admission showed a hemoglobin of 27% (3.9 gm. %); red blood cells 1,450,000 per cmm.; hematocrit 12.0%; M. C. V. 89.0 $c\mu$.; M. C. H. C. 25.8%; M. C. H. 32.5 micromicrograms; reticulocytes 1.2%; white blood cells 3900 per cmm., with a normal differential count. The platelets were greatly diminished in the blood smear. The icterus index was 5 units.

The patient received 2500 cc. of blood in the first 5 days, which raised her red blood cells to 3,070,000 per cmm. Figure 2 shows only the last 2 transfusions of 500 cc. of whole blood. Then during a period of 10 days, from March 26 to April 4, she received daily intramuscular injections of 1 cc. of Reticulogen-Lilly and 2 cc. of Liver Injection (Crude), U. S. P.-Wilson, containing 2 U. S. P. units (injectable) per cc., without significant reticulocyte response (Fig. 2). This therapy was immediately followed in a second period by the oral administration of Liquid Extract of Liver, U. S. P.-Valentine, 15 cc. 3 times daily, or the equivalent of 1 U. S. P. unit (oral) daily. On the 4th day of this treatment the reticulocytes began to rise and reached a peak of 13% on the 6th day. Thereafter the patient made rapid and dramatic clinical improvement, and her appetite and sense of well-being increased markedly. Epistaxes, which had previously been frequent, severe and difficult to control, subsided. The patient was discharged on April 15, 4 weeks after admission.

Since discharge the patient has been seen regularly at frequent intervals. She continued taking Liquid Extract of Liver, U. S. P.-Valentine until May 24, 2 months after entry, at which time blood examination showed a hemoglobin of 93%, red cells 4,140,000 per cmm., and white cells 8200 per cmm. The patient stated that she felt the best that she had ever felt and that she had an insatiable appetite. She had had no nosebleeds for a month and no purpura. The liver was no longer palpable.

CASE 3.* M. B., a 40 year old primipara at term, was admitted to the Obstetrical Service of the Boston City Hospital in labor on May 24, 1944. She had been in good health until about 3 months before admission, when her appetite, always somewhat poor, began to diminish noticeably. Anorexia increased progressively so that during the last 3 weeks she subsisted entirely on occasional egg-nogs. Two and one-half months before admission she developed increasing ankle edema, fatigability and pallor. Heartburn became distressing and on several occasions was associated with vomiting. Four weeks before entry her tongue became increasingly sore, especially at the margins. During the last week she had occasional mild nosebleeds and a few purpuric spots on her body. She had no paresthesias and no diarrhea at any time. She had never been anemic.

Physical Examination. Temperature 100° F.; pulse 120; respirations 20; blood pressure 120/80. The patient was a thin woman with waxen pallor, in active labor, appearing critically ill. There were 15 to 20 purpuric spots, averaging about 0.5 cm. in diameter, on the trunk and extremities. No icterus or adenopathy was present. The tongue was slightly reddened at the margins, but showed no papillary atrophy. There was a small ulceration on the left tonsillar pillar. The heart was normal except for a systolic murmur, heard best at the apex. The abdomen contained a protuberant mass consistent with a full term pregnancy. The liver and spleen were not palpable. There was obvious pitting edema of the ankles. Vibratory sense was intact; reflexes were normal.

Laboratory Findings. The blood examination on May 25 revealed: hemoglobin 37% (5.8 gm. %); red blood cells 1,720,000 per cmm.; hematocrit 15.8%; M. C. V. 91.8 $c\mu$.; M. C. H. C. 27.3%; M. C. H. 33.5 micromicrograms; reticulocytes 0.6%; white blood cells 3500 per cmm., with a normal differential count. The stained smear showed little variation in size and shape of the erythrocytes, with rare round but no oval macrocytes. Platelets were sparse and large. The neutrophils had toxic granules. The platelet count was 57,000 per cmm. The blood group was B, Rh positive. Icterus index was 4 units. The urine had a specific gravity of 1.020 and contained 1 plus albumin; the

* This patient was seen in consultation with Dr. C. D. Smith by Dr. Peter F. Weiss, who referred her to the Boston City Hospital. We are indebted to Dr. Weiss and to the Obstetrical Service of the Hospital for permitting us to study the patient.

sediment showed a few white blood cells, red blood cells and casts. Urine urobilinogen was positive to a dilution of 1:4. Gastric analysis on June 1 showed no free hydrochloric acid in the 3 cc. of fasting specimen, but after histamine there were 45 cc. of N/10 free hydrochloric acid per 100 cc. of gastric juice.

Course. On May 25, the day after admission, the patient was delivered by low forceps, under spinal anesthesia, of a normal female infant weighing 4 pounds and 2 ounces. Uterine bleeding was not excessive. During a period of 8 days the patient was given daily intramuscular injections of 5 cc. of a "special liver preparation" (Fig. 3). This material was a suspension-solution of Liver Extract-Lilly, N. N. R.,* containing 1.25 gm. per 5 cc., equivalent to about 2.5 U. S. P. units (injectable). During this course of therapy there was some decline in the red blood cell count, and the patient continued to appear gravely ill. From the 2nd to the 23d day she ran a fever ranging from 101° to 103° F. Although there was no specific evidence for a source of infection, sulfadiazine was given from the 4th to the 16th day but produced no appreciable clinical response. The pulse rate remained at 120. On June 2, 8 days after delivery, the patient's condition appeared so serious that parenteral liver extract therapy was abandoned. During a second period she was then given by mouth the same preparation that she had been given parenterally, receiving daily, in 3 doses, 51 cc., containing 12.75 gm. of Liver Extract-Lilly, N. N. R., or the equivalent of 1 U. S. P. unit (oral). Within 3 days after initiation of this therapy clinical improvement was noted coincidentally with a rise in reticulocytes, which reached a peak of 26.2% on the 7th day. This response was accompanied by a sustained rise in hemoglobin and red blood cell values. The patient's appetite improved strikingly and her edema disappeared. On June 16 the blood platelets had risen to 372,000 per cmm. The patient was discharged remarkably improved on June 21, 29 days after admission. Liver extract therapy was discontinued 1 month after discharge, since the hemoglobin had reached 84% and the red blood cell count was 4,100,000 per cmm. Following discharge the patient's brown hair fell out, and was replaced by a new growth of white hair. On February 20, 1945, 8 months after discharge, the blood count was still normal.

Case 4. S. G., a 76 year old housewife, was admitted to the Second Medical Service of Boston City Hospital on January 2, 1945. For 2 years the patient's appetite had diminished progressively so that her dietary intake for some time had consisted largely of oatmeal, tea and toast. She had never cared for meat and seldom ate it. For 4 weeks before admission she had had increasing fatigue, pallor and frequent vomiting. She gave no history of glossitis, neural symptoms, fever, jaundice or blood loss.

Physical Examination. Temperature 101° F.; pulse 120; respirations 22; blood pressure 110/45. The patient was a very pale, thin, white-haired elderly lady. No jaundice, purpura, or adenopathy was present. There were a few small punctate retinal hemorrhages in the right fundus. The tongue appeared normal. There was complete edentia. A few scattered moist râles were heard at both lung bases. The heart was normal except for a soft blowing systolic murmur of maximum intensity at the apex. The liver and spleen were not palpable. Neurologic examination was negative. There was no peripheral edema.

Laboratory Findings. The blood on admission showed: hemoglobin 25% (3.9 gm. %); red blood cells 1,230,000 per cmm.; hematocrit 13.8%; M. C. V. 112.2 $c\mu$.; M. C. H. C. 28.3%; M. C. H. 31.7 micro-micrograms; reticulocytes 2.6%; white blood cells 2800 per cmm. (76% segmented neutrophils, 10% band forms, 6% small lymphocytes, 1% large lymphocytes, and 7% monocytes). The stained smear showed moderate anisocytosis and poikilocytosis, with oval macrocytes and a few nucleated red cells and Howell-Jolly bodies. The platelet count was 98,000 per cmm. The icterus index was 7½ units. The patient's blood was group A, Rh positive. Blood non-protein nitrogen was 29 mg. %. Plasma albumin was 2.39 gm. %, globulin 2.14%, with a total protein of 4.53 gm. %. Blood Hinton test was negative. The urine showed a trace of albumin and a positive reaction for uribilinogen in a dilution of 1:32. Stools were guaiac negative. Gastric analysis showed 17 cc. of N/10 free hydrochloric acid per 100 cc. of gastric juice in the 25 cc. of fasting specimen, and 18 cc. after histamine.

* Kindly supplied by Mr. George B. Walden of The Lilly Research Laboratories.

Stool urobilinogen excretion studies showed the following values: 43.75 mg. per day for the 4-day period immediately preceding intravenous liver extract therapy (16th to 20th day), 182.7 mg. per day for the succeeding 4-day period (20th to 24th day), and 15 mg. per day for the 4-day period from the 36th to the 40th day.

Course. The patient's temperature gradually subsided and became normal on the 4th day after admission. She received ferrous sulphate, 4 grains 3 times daily, throughout her hospital stay. During a first 10-day period she was given daily, by intramuscular injection, 2 cc. of a suspension-solution of Liver Extract-Lilly, N. N. R., equivalent to about 1 U. S. P. unit (injectable). She showed no hematologic response to this therapy (Fig. 4). She was given a total of 900 cc. of whole blood during the last 3 days of this period and this raised her red blood cell count to 1,880,000 per cmm. During a second 10-day period she received daily, by mouth, crystalline B vitamins and accessory substances in the following amounts: thiamine chloride, 100 mg.; riboflavin, 100 mg.; niacinamide, 200 mg.; pyridoxine hydrochloride, 100 mg.; pyridoxal hydrochloride, 300 mg.; calcium pantothenate, 100 mg.; paraminobenzoic acid, 2 gm.; choline hydrochloride, 300 mg.; inositol, 200 mg.; biotin, 2 mg.; and *L. casei* factor, 1.3 mg.* These were mixed with 10 gm. of Borden's "Labco vitamin-free casein" and administered in 60 cc. of water. Again the patient failed to show any hematologic improvement. She was then started on a third period of therapy, receiving daily intravenous injections of 20 cc. of the supernatant from the suspension-solution of Liver Extract-Lilly, N. N. R., which was derived from 5 gm. of the powder and was equivalent in antianemic potency to about 10 U. S. P. units (injectable). The supernatant resulted from removal of 1.5% by volume of precipitate contained in the suspension-solution. This preparation was given in 250 cc. of 5% glucose in distilled water at the rate of 5 cc. per minute, in order to avoid causing flushing and dyspnea. On the 4th day of this therapy the reticulocytes began to rise and reached a maximum of 25.1% on the 6th day. Therapy was then discontinued because of the excellent hematologic response and because of the patient's dislike of venepunctures. On the day preceding the reticulocyte peak the patient developed clinical signs of bronchopneumonia. This diagnosis was confirmed by Roentgen ray and sulfadiazine was given for 2 days but produced no improvement. Penicillin was then given for 3 days. The patient improved clinically within 24 hours and remained afebrile thereafter. On February 2, 5 days after discontinuance of intravenous liver extract therapy, daily oral administration of 20 cc., the same amount, of the same preparation was begun. On the 3d day the patient's appetite began to improve noticeably and thereafter clinical improvement was rapid. There was a second rise of reticulocytes beginning on the 6th day and reaching a peak of 7.4% on the 9th day. This was accompanied by a rise in red blood cells to 3,480,000 per cmm. on February 27, 3 days before discharge on the 57th day of hospitalization. One month later the red blood cells had risen to 4,320,000 per cmm.

Conclusions. 1. Three cases of nutritional macrocytic anemia, 2 of which occurred during pregnancy, were shown under controlled clinical conditions to respond to orally administered liver extract immediately after failing to respond to multiple U. S. P. units (injectable) of parenterally administered liver extract. A fourth case responded immediately to parenterally administered liver extract when the dose was increased 10 fold.

2. As a result of a review of the literature, as well as of the observations on these 4 patients, it is apparent that at least two, and possibly three, types of nutritional deficiency are involved in nutritional macrocytic anemias, as evidenced by differences in therapeutic responses to various types of liver extracts:

a. Response to usual amounts of the most refined liver extracts administered parenterally. This is a large group, including Addisonian pernicious anemia and many cases of sprue and macrocytic anemia of pregnancy.

* "Folic acid" concentrate (*L. casei* factor 15% pure, prepared by fermentation methods) was obtained through the courtesy of Dr. Y. SubbaRow, Lederle Laboratories, Inc.; other vitamins were obtained through the courtesy of Dr. D. F. Robertson, Merck & Co., Inc.

b. Response *only* to orally administered crude liver extracts or to large amounts of certain relatively crude liver extracts given parenterally. This group includes certain instances of macrocytic anemia of the tropics, of anemia of pregnancy and of "refractory" anemias with megaloblastic bone marrow, as well as the cases presented here.

c. As to a third type, it is possible that the basic deficiency in our 2 patients who responded to orally administered crude liver extract differs from that in the 2 patients who responded to large amounts of more refined liver extract.

3. Since a distinction in therapeutic response to refined and crude liver extracts was first reported in anemic monkeys, and later in patients, by Dr. Lucy Wills and her associates, it is suggested that this effective principle in crude liver extracts and in autolyzed yeast be designated as "Wills' factor." It clearly differs from the principle in liver which is effective in Addisonian pernicious anemia and from the so-called extrinsic factor.

4. Observations on Case 4 indicated that Wills' factor is not identical with various pure components of the vitamin B complex and accessory nutritional substances, including biotin, *L. casei* factor and pyridoxal.

5. In instances of macrocytic anemia refractory to parenterally administered liver extract, especially those associated with pregnancy, it may be of practical therapeutic importance to test the efficacy of relatively crude liver extracts administered orally or, when suitably prepared, administered intravenously in large amounts.

6. The demonstrated therapeutic value of orally administered liver extracts in occasional patients with nutritional macrocytic anemia due to deficiency of Wills' factor should not obscure the well-established advantages of parenterally administered refined liver extracts in the treatment of the majority of patients with nutritional macrocytic anemias, especially those with Addisonian pernicious anemia.

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HOOKWORM INFECTION IN AMERICAN TROOPS IN ASSAM AND BURMA

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A clinical picture characterized by the acute onset of moderately severe gastro-intestinal symptoms associated with eosinophilic leukocytosis and hookworm infection was observed with increasing frequency in this hospital. It occurred predominantly in combat troops serving in North Burma during the summer and fall of 1944. It differed from the traditional clinical picture of hookworm disease chiefly in the abruptness of onset, the prominence of acute and sometimes disabling digestive symptoms and the lack of anemia. It presented problems of management, diagnosis and treatment of sufficient importance to prompt this report of 50 cases. These cases represent 50 consecutive instances of hookworm infection admitted or transferred to the gastro-intestinal and dysentery wards, and are therefore a selected group. Several hundred additional cases both with and without symptoms were seen on the general medical wards during the same period of time.

Clinical Features. Gastro-intestinal symptoms were predominant and were the primary reason for admission in well over half of these patients. In others who had been admitted for wounds or malaria, abdominal complaints became prominent during convalescence. In 7 of the group, abdominal symptoms had subsided by the time the diagnosis was made. The digestive symptoms began a few weeks to several months after the men arrived in North Burma. In many, a sudden onset of nausea, vomiting, abdominal pain and diarrhea occurred. In others, a more gradual onset of cramping and burning abdominal pains after meals were the initial manifestations. The nausea, vomiting and diarrhea tended to subside and to become intermittent.

Pain was the most prominent and persistent of the gastro-intestinal symptoms. Its location was usually epigastric but sometimes was periumbilical or in either the left or right upper quadrants. In general it was not localized to a single area but tended to be diffuse. The pain was variously described as burning, cramping, or aching. Its most definitive characteristic was its appearance immediately after meals. It was not relieved by eating. Certain foods, particularly fried greasy food and beer frequently made the pain worse. In a few instances, the epigastric pain somewhat resembled that of a peptic ulcer, coming intermittently and sometimes awakening the patient at night. The diffuseness of the pain, its usual occurrence immediately after meals, and the association with diarrhea were the clinical characteristics which aided in differentiating hookworm infestation from peptic ulcer. Results of gastro-intestinal Roentgen rays and stool examinations were the final criteria.

Diarrhea was a prominent and sometimes intractable symptom. The number of stools per 24 hours averaged about 6. Unless complicated by amebiasis, no blood, pus, or mucus were noted in the feces. The diarrhea was never associated with urgency or tenesmus. Amebiasis was always considered, but the prominence of upper abdominal symptoms, together with the absence of rectal symptoms and dysenteric

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stools with exudate, frequently enabled a differentiation to be made on a clinical basis.

The patients commonly maintained that they had developed "stomach trouble" from the C and K rations provided in the forward areas. However, when a hospital diet was provided their symptoms did not improve, and the anorexia, blamed on the unpalatable rations, persisted. This was in marked contrast to the healthy and vigorous appetites of other convalescent patients who did not have hookworm disease.

TABLE 1.—SYMPTOMS OF HOOKWORM INFECTION

	No. of cases in which a reliable history could be obtained	No. positive	% positive
Abdominal pain	49	42	86
Weight loss	36	29	80
Respiratory symptoms	41	29	70
Abdominal tenderness	48	32	66
Vomiting	46	27	58
Diarrhea	48	25	52
History of ground itch	42	12	28

The history of a skin rash which might be considered to be a "ground itch," caused by migration of the larvæ through the skin, was never volunteered. However, when the diagnosis of hookworm had been established and the patients specifically questioned on this point, about one quarter of them gave a suggestive history. The evaluation of this history was difficult because the incidence of other skin lesions among combat troops was high; miliaria, leech bites, and fungus infections were common, but some patients recalled having had intensely itching eruptions on the legs and thighs after sleeping in foxholes.

Respiratory symptoms which might be attributed to the passage of hookworm larvæ through the lungs were elicited in 70% of the patients. Many described a so-called "foxhole cough," well remembered because at night it gave away the soldier's position to the enemy. Again, the importance of this history has to be qualified. Upper respiratory infections were undoubtedly frequent, but the complaint of a "deep chest cold" without any preceding coryza or sore throat was unusually common. The cough often persisted from a week to several months after the last suspected exposure to hookworm larvæ.

The physical findings were not striking except for the almost universal loss of from 10 to 40 pounds in weight. In a few cases the weight loss was great enough to make the patients appear emaciated. It is true that weight loss among the combat soldiers in Burma was a common occurrence because of difficulties in obtaining palatable food, but the inability of the patients with hookworm disease to gain weight frequently persisted even after hospitalization when the diet was much improved in quantity and palatibility. A low grade fever of 99° to 100° F. which could not otherwise be explained, was present in a small number of hookworm patients. Upper abdominal tenderness was found in slightly more than half. Usually more than one tender area was found. In some patients, right upper quadrant tenderness was quite marked, and when in addition the liver edge was palpable, amebic hepatitis had to be excluded. Hyperactive peristalsis was frequently present. Three patients had bouts of otherwise unexplained urticaria while under observation.

One patient was admitted who gave such a straightforward and illuminating story about the development of his symptoms that the history is given in detail since the time relationship of the described symptoms is of interest in relation to the known life cycle of the parasite.

Case Abstract. A 25 year old white sergeant from an engineering unit was admitted

to this hospital on Nov. 27, 1944. His chief complaint was diarrhea of 2 weeks duration. This consisted of 6 to 8 watery stools a day without any blood or mucus. It was associated with abdominal pain which had never been violent or attended by urgency or tenesmus. In addition to the diarrhea he complained of burning upper abdominal pain, vomiting, and anorexia. These symptoms suggested hookworm infection. With this in mind the patient was questioned about rash and cough. He then gave this history: On Oct. 27, 1944, a month prior to admission he crawled under a mobile truck-crane and worked with his back on the ground for more than an hour. His shirt tails were outside of his trousers thereby exposing his skin directly to the ground. In addition he had a large hole in his shirt which exposed to the ground an area of skin between his scapulæ. He experienced sensations which he interpreted as being due to having straw in his shirt. He removed his shirt and shook it out. This did not relieve the itch. He was so disturbed that night that he reported to his dispensary. The medical technician told him that he had a rash resembling poison ivy. The itching eruption continued for another 2 days and then subsided. During the night of Oct. 30, 1944, the fourth day after working under the truck, the patient was awakened with a deep cough. He volunteered that the symptoms were different from an ordinary chest cold and this made him fear that he might be getting pneumonia. He again reported to sick call and was told that he had bronchitis. The cough persisted for 12 days. At noon on Nov. 12, 1944, 14 days after exposure the patient, having eaten lunch, suddenly felt bloated and began to vomit. From then on he had burning, cramping abdominal pain which was made worse by eating. He vomited frequently. On Nov. 16, 1944 diarrhea began. His gastro-intestinal symptoms did not respond to the usual dispensary remedies and he was referred to this hospital for further studies. Examination revealed an unhappy and haggard, but well developed and nourished soldier. Abdominal palpation disclosed a diffuse mild upper abdominal tenderness and two fairly localized areas of more marked tenderness, one in the left upper quadrant and the other in the left lower quadrant of the abdomen. No rash or signs of pulmonary disease were present. A clinical diagnosis of hookworm disease wae made. On the following day examination of a direct saline suspension of stool revealed hookworm eggs. Blood studies revealed 16.2 gm. of hemoglobin and a total leukocyte count of 13,100 (neutrophiles 11%, lymphocytes 21%, monocytes 2%, and eosinophiles 66%). Gastro-intestinal Roentgen ray studies were reported by Captain George P. Keefer to show a small intestinal pattern characteristic of hookworm disease.[3] The chest Roentgen ray was negative. The patient was given 3 cc. of tetrachlorethylene and his stools were collected for the next 24 hours. A total of 64 adult hookworms was recovered: 43 were *Ancylostoma duodenale* and 21 were *Necator americanus*. A week later the patient was symptomatically much improved. A few eggs were seen in a concentrated specimen though none was found on direct examination. The leukocyte count had dropped to 8000 of which only 30% were eosinophiles. One gm. of hexylresorcinol was then administered. At the end of another week a few eggs were still found in the feces by the concentration technique. The leukocyte count was then 6400 with 25% eosinophiles and the patient had become symptom free. He was then given a third vermifuge consisting of 3 cc. of tetrachlorethylene. Four and 10 days later concentrated specimens were negative for hookworm eggs. The blood count revealed 6200 leukocytes with 54% polymorphonuclear leukocytes, 40% lymphocytes, and 6% eosinophiles. The patient was discharged to duty on Jan. 12, 1945, completely recovered.

This patient's history gave a remarkably clear picture of the time relationships between the entrance of the larvæ into the skin and the appearance of the symptoms resulting from the migration of the parasite through the lungs to the small intestines. In a number of other patients a similar clearcut sequence of symptoms could be uncovered by detailed questioning. In most instances where the duration of exposure extended over a period of weeks, the patients could remember when their gastro-intestinal symptoms began but the date of onset of itching skin eruption and cough could not be recalled.

Laboratory Studies. The definite diagnosis of hookworm infection depended

upon the demonstration of the eggs in the stool. For this purpose the direct examination, even when repeated several times, was not a satisfactory procedure. It was only after the zinc sulfate flotation centrifugation technique[2] was adopted, that the eggs could be found consistently. This difference between the two methods is amply demonstrated by the following results.

Of the 50 patients considered in this report, the stools of 42 were first examined by the direct method. The diagnosis was established after repeated examinations in only 24 (57%) of the patients. Only 9 (21%) had positive stools on the initial examination. On the other hand 35 of the patients had stools examined by the concentration method. In all 35, hookworm eggs were found after not more than 3 stool examinations and in 30 (86%) they were demonstrated on the initial examination. A positive report was obtained from the laboratory on the first trial in 8 of 10 patients in whom the direct method had failed from 5 to 10 times. In all, of 145 stools examined by the direct method only 24 (17%) were positive whereas of 46 stools examined by the concentration technique 38 (82%) were positive for hookworm eggs.

Charcot-Leyden crystals in the stools are regarded as an abnormal finding which may be indicative of intestinal parasitism. Where specifically looked for, these crystals were found in the stools of 18 of 27 patients.

In 14 of the patients, 24-hour stools were collected following vermifuge and examined for adult hookworms. In specimens from 13 patients, the number of adults recovered by detailed, systematic study averaged 37. In a specimen from the remaining patient, over 100 adults were identified. On the basis of these examinations and those for eggs, the worm burden was considered small. No egg counts were performed, but the number of eggs encountered by the direct and concentration methods of examination was considered further evidence for assuming a small worm burden. *A. duodenale* was identified in 12 of the 14 specimens studied in this manner. Two of these in addition had *N. americanus*, and the remaining 2 had *N. americanus* only.

Among the striking features observed, was the degree of eosinophilia. Its discovery was the chief reason for the examination of the stool for eggs in many instances. The maximum eosinophilia recorded was 70%, with a total leukocyte count of 41,000. The more usual finding was an eosinophilia of 40% with about 15,000 leukocytes. Fluctuation in both the total leukocyte count and the percentage of eosinophiles was very common. The earliest time for appearance of eosinophilia after the suspected date of infection in these 50 patients was 30 days. The eosinophilia persisted in most of them to a diminished degree for weeks and months despite frequent vermifuges.

Anemia was neither marked nor constant, a hemoglobin of 12 gm. or below being present in but 6 patients in this group. A severe wound, falciparum malaria or scrub typhus were at least partly responsible for the anemia in 3 of these 6.

Gastro-intestinal Roentgen ray studies were made in 42 of these patients.[3] In 34, abnormalities of the small intestinal pattern were noted. These varied from mild rugal thickening and increased tone to a very marked change in the appearance of the bowel. Tenderness was elicited over involved bowel loops. The abnormalities were most prominent in the jejunum but the distal duodenum was also involved. In 14, the roentgenologist was able to make a presumptive diagnosis of hookworm infestation. In 4 patients the deep broad indentations in the column of the barium made by the thickened rugal folds of the jejunum were very striking. Captain George P. Keefer suggested the term "cogwheel" pattern which describes its appearance very well. Mild degrees of this type of gastro-intestinal Roentgen ray change were reported in patients in whom hookworm infection was not demonstrated. However, when the alterations were marked, errors were infrequent. Some-

times the roentgenologist correctly suggested the diagnosis before it had been suspected on clinical grounds. The roentgenologic manifestations were remarkably persistent. In some they outlasted the symptoms.

Epidemiology and Incidence. Most of these patients were combat soldiers. The remainder were service troops living under field conditions. These men had served in areas lacking adequate sanitation, sleeping in foxholes partially filled with mud and water and bathing in rice paddies. The opportunity for exposure to the larvæ of hookworm was great. It is probable that all of these patients contracted hookworm infection in this theatre. Only one-fifth of the group had lived in the "hookworm belt" in the United States, and *A. duodenale*, a species uncommon in the United States, was recovered in high percentage. We have no reliable data on the actual incidence of hookworm infection among the troops who served in the forward areas, but it must have been considerable.

Treatment. The routine treatment consisted of a fat-free supper followed by a purge of 60 cc. of a saturated solution of magnesium sulfate. The next morning breakfast was omitted and 3 cc. of tetrachlorethylene in gelatin capsules were administered. A second purge was given 2 hours later.

An interval of 3 weeks was allowed to elapse between treatments. The second or subsequent vermifuge was usually tetrachlorethylene, although carbon tetrachloride (3 cc.) or hexylresorcinol (1 gm.) was sometimes substituted especially in resistant cases. The drugs were never given on the same day in combination.

No alarming symptoms appeared after the use of any of these drugs. A mild intoxication with manifestations similar to those produced by ethyl alcohol usually appeared about 20 minutes after tetrachlorethylene or carbon tetrachloride was administered.

When we first began to treat these patients, it was hoped that a single vermifuge would be sufficient. However, it was soon found that the persistence of symptoms, associated with many eggs in the stools and a continued leukocytosis with eosinophilia, necessitated repeated treatments. In the absence of symptoms the continued presence of a few eggs or a slight eosinophilia was not considered an indication for repetition of the vermifuge. In all, the 50 patients received 110 doses of anthelmintic drugs.

Results of Treatment. The period of observation extended from a minimum period of 1 month when the results of treatment were favorable, to as long as 6 months in resistant cases. The patients have been analyzed from the standpoint of symptomatic improvement, the disappearance of eggs from the stools, and a reduction of the leukocytosis and eosinophilia.

A. *Symptoms.* At the time that treatment was instituted 43 patients had abdominal symptoms. At the conclusion of the period of observation, 9 patients (20%) were completely relieved, 23 (55%) were improved, and the remaining 11 (25%) denied any decrease in their symptoms. The complaints of anorexia, vomiting and diarrhea were most readily relieved. The cramping pain after meals was the slowest to disappear and was the symptom which persisted in those who were unimproved. In general the more stoical and emotionally stable individuals improved rapidly or were cured by 1 to 3 treatments. In most of these who were not improved after repeated vermifuges, the past history revealed either prior gastro-intestinal complaints of a functional nature, nail biting, enuresis or some other manifestation of emotional instability. Eventually after prolonged study, the unimproved group (11 patients) were reassigned from combat duty to other tasks. In every instance, psychoneurosis was considered the primary reason for reassignment and no patient was reassigned because of the resistant hookworm infection.

B. *Stool Findings.* One week or more after the last vermifuge 34 patients had

specimens examined by the concentration method. Of these 20 (63%) still showed hookworm eggs, although they had been treated an average of twice each.

C. *Eosinophilia.* The eosinophilia, as has been pointed out previously, varied considerably from count to count, but in general it decreased after the administration of a vermifuge. The degree of eosinophilia dropped from an average maximum of 13,700 leukocytes with 34% eosinophiles to 9600 leukocytes with 21% eosinophiles. In only 3 patients did the eosinophilia drop below 4% following treatment.

Discussion. The clinical picture of abdominal pain, vomiting and diarrhea in patients with a high eosinophilia and hookworm eggs in the stool occurred with a frequency to make us believe that these cases were true instances of acute hookworm disease. This is not the usual symptomatology ascribed to hookworm infection in which anemia dominates the picture, nor to the lesser infections where the symptoms are mild or non-existent. A number of factors may have influenced the picture of the disease as we have seen it. First, the patients were presumably previously uninfected individuals who had been exposed to the parasite over a short period of time. How much of their symptomatology may have represented a local allergic manifestation and how much may have been due to local irritation produced directly by the worms in the upper small intestine is a matter of conjecture. The very high eosinophilia in these patients suggests a considerable reaction to foreign protein. The circumstance of a previously unexposed group of young adult white males being exposed to *A. duodenale* may in itself, in large degree, explain the observations. A striking feature was the presence of incapacitating abdominal symptomatology occurring with a small worm burden. *A. duodenale* is recognized as causing a more severe form of hookworm disease than *N. americanus* and to produce symptoms with a smaller number of adult worms.[3] A second possible factor has been the high incidence of intercurrent disease and battle wounds. Relapsing vivax malaria, amebiasis, bacillary dysentery, typhus, and cutaneous diphtheria occurred in some of these patients. Debility from these diseases may have played a part in exaggerating the gastro-intestinal symptoms. A third factor was the situation in which the disease was contracted. In many instances the symptoms appeared shortly after the soldiers arrived in combat for the first time. The emotional stress, the restricted diet, and the physical fatigue undoubtedly caused an exaggeration of gastro-intestinal symptoms. Furthermore, once the patient was evacuated to the rear, either a conscious or subconscious effort to escape further combat duty may have increased the severity of the symptoms in many. The decision has been difficult to make as to whether a given individual was (*a*) malingering; (*b*) having symptoms chiefly as the result of intestinal parasites, or (*c*) whether his complaints were aggravated by, or entirely due to a psychoneurosis. When after repeated treatments a soldier continued to complain of abdominal pain and had, in addition, eggs in the stools and a persistently abnormal gastro-intestinal Roentgen ray pattern, he became a difficult medical problem. The weight of our experience indicated that after one or two vermifuges, hookworm infection should no longer be disabling. Every effort was made to minimize to the patient the importance of a few hookworm eggs in the stool and to discharge him to duty as soon as he became free of symptoms.

The difficulties in demonstrating hookworm eggs in the stools by the direct method were great. The effectiveness of the zinc sulfate flotation-centrifugation method for finding hookworm eggs should be stressed. It was not at once presumed that the clinical picture observed could have been produced by the small worm-loads noted, but only after thorough consideration of many detailed clinical histories. Until this method was put into routine practice there were a large number of patients on the wards of this hospital

with unexplained abdominal pain associated with eosinophilia. Even with the help of this improved laboratory technique, a small number of patients in whom we suspected hookworm infection had repeatedly negative stools. In a few patients not included in this study who had consistently negative stools prior to treatment, adult worms were recovered following the vermifuge. In specimens from other patients, eggs were not demonstrated until a vermifuge had been given. In many, eggs were found only intermittently.

A period of time necessarily elapses from the entrance of the larvæ through the skin and the appearance of eggs in the stools. In 1 patient eggs were demonstrated as early as 1 month after the probable date of exposure. In a number of patients, difficulties in proving the presence of the parasite may have been due to the fact that not enough time had passed for full development of the adult worms. We suspected that the eosinophilia appeared before eggs were passed in the feces. No data concerning this point are available in this series of patients.

The drugs used in the treatment of this group of patients: tetrachlorethylene, hexylresorcinol, and carbon tetrachloride probably removed the majority of the worms. By so doing, these anthelmintics probably prevented an anemia and relieved patients of most of their symptoms. In civilian practice this has apparently sufficed, but in military practice a more effective vermifuge is desirable. However, it is stated that *A. duodenale* infections are more refractory to therapy than *N. americanus* infections.[4] In the first place a drug which could have eliminated all the hookworms in a single treatment would have prevented the prolonged hospitalization required in this group of patients in whom 2 and 3 treatments were necessary. Since 3 weeks were permitted to elapse between each treatment a soldier had to be hospitalized at least 6 weeks to be treated 3 times. The second reason why a more effective drug is needed is that the persistence of hookworm eggs in the stools erects a stumbling block which impedes the rapid recognition of functional disease or malingering and therefore delays the proper disposition of patients. It is agreed, however, that for practical purposes, the aim of treatment with drugs at present available, need not be the removal of all adult worms.

Some mention should be made of strongyloidiasis because it may produce a very similar clinical picture of abdominal pain and diarrhea with a very marked eosinophilia. The gentian violet treatment, both with enteric-coated tablets and duodenal instillation of the drug, has been ineffective in eliminating this parasite. Enough symptomatic improvement occurred over a period of 2 or 3 weeks in most instances so that the patients were returned to duty even though they were still passing occasional rhabditiform larvæ.

In conclusion in presenting this group of cases of hookworm infection we do not wish to imply that we believe all soldiers who acquire the infection have the more acute symptoms which have been described. Many additional cases of entirely asymptomatic hookworm infection have been discovered in patients who were admitted for some unrelated disease. It is our belief that a large number of men who have lived under combat conditions in this theatre have hookworm infection. This group of 50 patients includes soldiers who have been most heavily infected, those who have reacted most violently to the presence of these parasites, and those in whom any illness might precipitate a psychoneurosis.

Summary. 1. In this theatre, a relatively acute syndrome of abdominal pain, anorexia, nausea, vomiting, diarrhea, and weight loss was observed in soldiers who had a marked eosinophilia and hookworm infection. The symptoms were believed to have begun within a few weeks after the penetration of the skin by the larvæ and to have persisted for several months in a diminished degree.

2. *A. duodenale* infections predominated. The severity of the clinical symptoms was considered disproportionate to the worm burden, which was judged to be small.

3. The initial direct stool examination revealed hookworm eggs in only 20% of the patients. By repeated direct examinations it was possible to establish the diagnosis in a little more than half. The zinc sulfate flotation-centrifugation method proved a rapid and efficient means of finding eggs in the stool, increasing the number of positive examinations approximately four-fold.

4. Tetrachlorethylene, carbon tetrachloride, and hexylresorcinol have not proved entirely satisfactory as vermifuges since they were unable to eliminate all the parasites even when given repeatedly. This has resulted both in prolonged hospitalization and in delay in the proper disposition of those patients who had little inclination to return to duty.

5. Most textbook descriptions of hookworm disease present a clinical picture dominated by anemia and malnutrition. In the light of these observations, it is clear that hookworm disease in white young adults may be an acute enteritis before anemia and malnutrition develop; the diagnosis can often be suspected clinically at this stage, on the basis of the phenomena described in this report.

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RUTIN: A NEW DRUG FOR THE TREATMENT OF INCREASED CAPILLARY FRAGILITY

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Rutin is a crystalline glucoside of quercetin derived from buckwheat leaves and blossoms, tobacco leaf, rue herb, tomato stems, white hydrangea,[3] yellow pansies, the leaves of the common elderberry, and many other flowers and leaves. Chemically, it is a rhamnoglucoside of quercetin, thus being a derivative of flavone. Flavone, in turn, is a derivative of gamma-pyrone and from it are formed various yellow dyestuffs. Citrin is an impure mixture of two flavone glucosides, hesperidin and eriodictyol, which, in association with vitamin C, have been thought to regulate vascular permeability. This factor, supposed to regulate capillary permeability, also has been named vitamin P.[1]

Citrin was prepared by Szent-Györgyi in 1936 from lemons or paprika and he found that this compound corrected experimentally produced increased capillary fragility in guinea pigs. However, the crystalline glucosides, hesperidin and eriodictyol, have been found to be physiologically inert, while the active compound in these crude glucosides never has been discovered. Although it never has been proven, the probability exists that the active substance is rutin.

The close relationship between hesperidin and rutin can be seen by inspection of their structural formulæ:

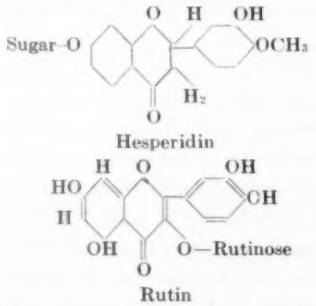

Fig. 1.—Structural formulas of hesperidin and rutin.

The drug, rutin, shows neither acute nor chronic toxicity to laboratory animals and, on this basis, its effects were tried on man. It was found that patients treated with daily doses of rutin for as many as 16 months showed no toxic effects attributable to the drug.[4]

Material. The rutin used in this study was prepared by the Eastern Regional Research Laboratory of the U. S. Department of Agriculture. It is prepared by alcohol percolation from green buckwheat. The drug is a bright yellow powder consisting of needle-shaped crystals melting at 192 to 196° C. and decomposing at 214° C. It is sparingly soluble in water: 0.13 gm. per liter at 20° C. and more in boiling water. It is soluble in methanol, ethanol, pyridine, acetone, ethyl acetate and alkalies. It is insoluble in chloroform, ether and hydrocarbons. It can be hydrolyzed with dilute acids to quercetin, glucose and rhamnose.[2]

Rutin is superior to crude hesperidin in that the rutin may be purified by recrystallization, thus making assay and accurate dosage possible.

The patients studied were taken from the private practice of the author, with one exception, and consist mainly of hypertensive subjects who had been studied according to Griffith's[6] method. He has suggested "that an abnormal condition of the capillaries might be a factor in the production of certain of the vascular accidents which sometimes occur in cases of hypertension."[5] The method also was used as a control of vascular complications in the thiocyanate therapy of hypertension and in several cases of unexplained bleeding associated with increased capillary fragility.

Method. *Capillary fragility* was measured by Gothlin's technique. A description of this method taken from Griffith and Lindauer[5] follows:

"Technique of the Test:—(1) Mark off a circular area, 6 cm. in diameter, in each antecubital area. Mark off all blemishes and marks in this area that might later be

confused with petechiæ. (2) Place a standard blood pressure cuff about each arm, and maintain in each a pressure of 35 mm. of mercury for 15 minutes. Lower the pressure, and count and mark all petechiæ within the two circular areas, using a good light and a magnifying lens of 5 D or its equivalent. (3) One hour or more later, repeat, using a cuff pressure of 50 mm. of mercury.

"*The Petechial Index* is calculated as follows: To the number of petechiæ occurring at 35 mm. of mercury multiplied by 2, add the additional number occurring at 50 mm. Based upon the Petechial Index, capillary fragility is regarded as: (*a*) normal, if the Index is 8 or less, (*b*) increased (abnormal) if the Index is 13 or more, and (*c*) borderline, but probably abnormal, if the Index is 9 to 12. In order to save time, the second stage can be omitted under the following conditions: (1) The number of petechiæ after the first stage is 2 or less. Such persons may be considered normal. Usually, but not invariably, the person is normal who has 3 petechiæ after the first stage. (2) If 6 or more petechiæ appear after the first stage, the subject may be considered abnormal. (3) The test is a repetition, and may be compared with the corresponding first stage of an earlier test. Repetition is less than 3 weeks, however, is unreliable in any case. The second stage should always be done if the fragility is being tested in a subject for the first time and there are 4 or 5 petechiæ after the first stage. It should also be done in most cases when the number after the first stage is 3."

Dosage. The dosage of rutin used was 20 mg. 3 times daily. A few patients who did not respond to this dosage were given 40 mg. 3 times daily.

In each case of the hypertensive and thiocyanate subjects a complete history was taken and a detailed physical examination including chest fluoroscopy, electrocardiogram, eye ground examination, and kidney function studies, was done. This was simply a matter of selection to rule out any other serious organic disease. The series comprises 32 cases divided as follows:

1. Hypertension—24.
2. Thrombopenic purpura—1.
3. Pulmonary hemorrhage with increased capillary fragility—2.
4. Complete heart block with retinal hemorrhage—1.
5. Nuclear lesion of the eighth nerve—1.
6. Increased capillary fragility due to drugs—3.
   (*a*) Sulfadiazine—1.
   (*b*) Gold salts—1.
   (*c*) Acetyl salicylic acid—1.

GROUP 1. The first group consisted of 24 hypertensive subjects. Of these, 13 were persons who showed increased capillary fragility ranging from an index of 10 to 20 (average 16). The remaining 11 consisted of patients with hypertension being treated with potassium thiocyanate. For convenience these are being described in subgroups. (*a*) In the group of 13 cases repeat determinations of capillary fragility were made within 3 to 4 weeks after the institution of rutin therapy. Observation has ranged from 6 weeks to 9 months. The 13 cases showed an improvement within the initial 3 weeks period. Two of these became normal within this period, while all became normal within 12 weeks and have remained normal to the present time. None of the group has had any demonstrable vascular accident during the period of treatment. (*b*) There were 11 patients being treated with potassium thiocyanate for hypertension. Seven of these cases with capillary fragility originally normal were given rutin and thiocyanate coincidentally and the Gothlin Index remained normal. Three cases with capillary fragility initially normal were given potassium thiocyanate alone and developed an increased fragility. Rutin was added to the treatment and capillary fragility became normal. One case with an increased index was given rutin and followed by thiocyanate. The index became normal and remained so.

GROUP 2. One case of essential thrombocytopenic purpura was treated with rutin without benefit for a period of 2 weeks, which may have been an insufficient interval. The patient was too ill to permit a longer trial, and splenectomy was performed with subsequent recovery.

GROUP 3. Two cases showed recurrent pulmonary hemorrhages of variable amounts. Both were studied extensively including bronchoscopy and bronchograms with no demonstrable pulmonary lesions being found. Capillary fragility in both cases was increased. Because of the interesting features of these cases, brief summaries of their clinical features are presented.

Case Abstracts. CASE 1. J. B., aged 38, a white female housewife, complained of bleeding from the mouth for 3 months. The bleeding was about 1 drachm at a time and occurred 3 to 4 times a week. Bronchoscopy revealed nothing positive except a small suspicious area covered with blood in one bronchus. Bronchograms showed no pathology from a Roentgen ray standpoint. Blood examination: 3,930,000 erythrocytes; 13 gm. hemoglobin; cell volume of 41%; 255,450 platelets; 4300 leukocytes (55% neutrophils, 4% eosinophils and 41% lymphocytes). Sedimentation rate was 21 mm. in 1 hour by the Wintrobe method; prothrombin time was 30 seconds, or 83% of normal. Gothlin Index was 17. Treatment was started with 20 mg. of rutin 3 times a day. At the end of 3 weeks the Gothlin Index was 6 and bleeding had ceased. During the second 3 weeks there was no bleeding and there has been none reported after 6 months.

CASE 2. M. S., aged 20, a white female, was seen with a complaint of massive pulmonary hemorrhages over a period of 3 years. The first of these followed 1 week after an automobile accident in which she received a bump on her sternum. There have been 6 recurrences in which the bleeding was profuse. The patient was studied in 1942 by Dr. L. H. Clerf who reported as follows:

"At that admission it was thought that the blood was vomited. X-rays of the gastrointestinal tract and of the chest were negative. Esophagoscopy was negative. Bronchoscopy was negative except that some blood tinged secretion was seen coming from the right lower lobe bronchus, but this was observed on only one occasion and no apparent cause could be discovered. Hematologic studies revealed no blood dyscrasia, except a low hemoglobin and red blood cell count following a recent hemorrhage. Iodized oil was instilled to determine if there was bronchiectasis. Several of the sub-divisions of the right lower lobe bronchus revealed incomplete filling due apparently to the presence of some obstruction, particularly clotted blood. The remainder of the tracheobronchial tree appeared normal."

In April, 1945, she was studied in the Medical Ward of the University of Pennsylvania Hospital (service of Dr. O. H. Perry Pepper). Here, 3 days after admission, the patient coughed up 80 or 90 cc. of bright red blood. Immediate examination by an otolaryngologist showed the blood was coming from the respiratory tract, and not from the nose, mouth or larynx. Bronchoscopy showed blood in the trachea, but no source of bleeding. The patient was bronchoscoped 3 times and lipiodol twice was instilled and the entire tracheobronchial tree mapped out by Roentgen ray, but no lesion was discovered. Physical examination was negative. Other studies included: Platelets, normal, 202,000 in number. Prothrombin time 95% of normal. Bleeding and clotting times both normal. Blood count: hemoglobin 84%; red blood cells, 4,600,000; white blood cells, 6000 to 10,300, with a normal differential count: neutrophils, 63 to 68%; lymphocytes, 19—31%; mononuclears, 9%; eosinophils, 3%; basophils, 1%. Serum proteins, 6.8 gm.; serum cholesterol, 203 mg. %, fasting blood sugar, 82 mg. %; blood urea nitrogen, 10 mg. %; BMR + 5%. Sedimentation rate was 35 mm. per hour, which, by the method used, was slightly increased. Serology was negative. Repeated urinalyses showed occasional white cells, but nothing else. The tuberculin test was barely positive.

Fragility studies were done by the author and capillary fragility, measured by the method of Gothlin, was increased, 10 petechiæ appearing after the first stage of the test. The patient was placed on rutin, 20 mg. t.i.d., and discharged May 19, 1945. She was readmitted July 11, 1945, and stated that she had had no more bleeding episodes since the last admission. Capillary fragility was normal, only 2 petechia appearing after the first stage of the Gothlin Test. Chest Roentgen ray and bronchoscopy were repeated, and were unchanged. The rutin was continued. The patient volunteered the statement that she felt better than she had felt for 3 years.

GROUP 4. One patient, with complete heart block and a blood pressure of 280/160, suffered a massive hemorrhage of the retina resulting in blindness except for light perception. Capillary fragility was 20 and there were numerous dime-size areas of ecchymosis of the extremities. This patient was given 20 mg. of the drug 3 times a day with reduction of the index to normal and its maintenance within normal limits after 5 months. An occasional ecchymosis is seen but there has been no retinal involvement of the good eye. There has been no effect on blood pressure.

GROUP 5. Another case presented with evidence of a small nuclear lesion of the eighth nerve. The Gothlin Index was 14 which was reduced to 2 after 3 weeks of rutin therapy.

GROUP 6. A group of 3 cases showed increased capillary fragility as a result of drug sensitivity. One had a diffuse purpuric rash as a complication of sulfadiazine. Bleeding from the gums and urinary tract also occurred. The drug immediately was withdrawn and therapy with rutin started. Within 3 days external bleeding stopped and no new purpuric spots were noted. A similar experience was noted after the use of gold salts for rheumatoid arthritis. Whether the withdrawal of the drug or the use of the rutin was the effective therapy is a question. A third case seemed to have increased capillary fragility from the use of aspirin. The coincidental use of rutin with aspirin did not improve the state of the capillaries, for, on one occasion, a single dose of 20 gr. of aspirin, taken independently by this patient, while on rutin, produced a severe allergic reaction marked by urticaria, vasomotor rhinitis, and many small capillary hemorrhages about the shoulders and arms. Capillary fragility determination showed the index to be 4 which is well within the limits of normal. From this study it would seem that the reaction of the capillaries in the group termed "allergic purpura" is different from that in the group showing increased capillary fragility and one can infer that the value of rutin could be questioned in the allergic cases.[7]

Summary. 1. The drug, rutin, was used in a series of cases showing increased capillary fragility under a variety of circumstances.

2. The drug is non-toxic for man.

3. Results are reported in the following cases:

A. *Hypertension.* A group of 24 c[ases] divided into 2 subgroups was cho[sen]. Subgroup (*a*) consisted of 13 cases wi[th] increased capillary fragility all of which were improved with rutin. Subgroup (*b*) consisted of 11 cases treated with thiocyanate and rutin; 7 of these had normal fragility which was maintained by the prophylactic use of rutin given coincidentally with thiocyanate; 2 cases on thiocyanate alone developed an increase in the Gothlin Index which became normal following treatment by rutin; 1 case originally showing an increased index was given rutin followed by thiocyanate when the index became normal and was maintained subsequently at a normal level.

B. Two cases of *pulmonary hemorrhage* of undetermined origin, with increased capillary fragility, were given rutin with cessation of bleeding and a return to normal of the Gothlin Index.

C. Three cases of increased capillary *fragility due to drug reactions* were treated with uncertain results.

D. One case of small *hemorrhage into the eighth nerve nucleus*, and 1 with *complete heart block and retinal hemorrhages* were studied. Both showed increased capillary fragility which returned to normal following medication by rutin.

Conclusions. The drug, rutin, has an action on capillaries, which action appears to be equal to or possibly superior to that shown by members of the hesperidin group. This action is demonstrated in the cases here reported showing increased capillary fragility. With due regard to the relatively small number of cases and short periods of observation, it is reason-

able to conclude that the drug appears to be of value in: (1) preventing vascular accidents in patients with hypertension; (2) maintaining normal capillary fragility, and hence the avoidance of vascular accidents in patients being treated with thiocyanate; (3) controlling pulmonary bleeding when no other cause is apparent.

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ACUTE ETHYLENE GLYCOL POISONING

A CLINICO-PATHOLOGIC REPORT OF EIGHTEEN FATAL CASES

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THE ingenuity displayed by some in finding substitutes for alcoholic beverages is remarkable, as evidenced by this report which deals with 10 soldiers who drank lethal quantities of anti-freeze solution of the ethylene glycol (Prestone) type. The opportunity to study such a group is seldom to be had, if one may judge from the paucity of literature on human cases. Nearly all of the papers on ethylene glycol poisoning have been concerned with experimental toxicology and, even in these, descriptions of pathologic changes have been brief and incomplete.[3,4,7,8,9]

Ethylene glycol ($HO.CH_2.CH_2.OH$) is a colorless, odorless liquid which evolves heat when mixed with water. It possesses a rather pleasant, bitter-sweet flavor and when swallowed imparts a warming sensation to the tongue and esophagus. After ingestion the compound is oxidized, at least in part, to oxalic acid; urinary oxalates are increased and crystals of calcium oxalate are deposited in the kidneys. The minimum lethal dose for human beings has been estimated at about 100 cc.[5]‡

The most spectacular postmortem finding in acute ethylene glycol poisoning, both in man and experimental animals, is the extensive oxalate "crystallosis" of the renal tubules. Observations such as Brekke's[2] in attributing recovery in his 2 cases to unilateral decapsulation of the kidney have led to the belief that death results from renal damage, even though the outstanding symptoms are indicative of central nervous system damage. We suggest that the operation did not contribute much to the survival, but rather that the patients had consumed a sublethal amount of ethylene glycol.

Boemke[1] is the only author who has described significant structural changes in the brain, even though he has not been inclined to attach much importance to them. He noted generalized hyperemia of the brain and leptomeninges in 2 of his 4 cases; small ring hemorrhages in the brain stem and cerebral white matter were seen in 1 of these, as well as a perivascular cell infiltrate in both gray and white matter of the cerebrum. Boemke regarded the cellular reaction as residual from a previous infectious disease, such as typhus, and the hemorrhages as due to action of the chemical to increase the permeability of already weakened vessel walls. The brains in our series of previously healthy soldiers displayed all gradations of change, from simple hyperemia and edema to severe chemical meningo-encephalitis, and Boemke's explanation is thereby refuted.

Clinical Data. Due to the urgent need for treatment, the short course, and the field conditions under which most of the patients were observed, clinical notes and laboratory studies were meager and in 2 cases completely lacking.

The patients were all males ranging in age from 20 to 33 years; 6 were white and 4 Negro. Death occurred in from 22 to 44 hours after ingestion of the fluid except in 1 instance. This man drank several glasses of "vodka" and, although a companion§ "passed out" 10 hours later, the

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‡ The amount consumed was 200 cc. in 1 of our cases, unknown in the others.
§ We were unable to trace this case.

onset of his symptoms was delayed for 2 days and he lived 3 days thereafter. The men were not observed in the initial stages of the intoxication, but 1 had a history of early vomiting which subsided, and 2 continued to vomit until death.

or 12 hours, probably within the first few hours. The single exception was the soldier already mentioned whose symptoms were delayed; coma developed late after a period of restlessness and delirium. Two of the patients had incontinence of urine and feces; 4 had severe convulsive

TABLE 1.—COMPARISON OF CERTAIN CLINICAL DATA WITH CENTRAL NERVOUS SYSTEM LESIONS IN 10 CASES OF ACUTE ETHYLENE GLYCOL POISONING*

Case No.:	I	II	III	IV	V	VI	VII	VIII	IX	X
Age	20	28	21	21	30	30	..	33	24	20
Race	W	W	W	W	N	N	N	W	N	W
Sex	M	M	M	M	M	M	M	M	M	M
Lived (hrs.)	..	44	24	24	28	22	41	120	43	32
Temperature (°F.)		98–102	†	†	93	93	93–102			
Pulse		96	120	130	64	54	42–108			
Respiration			30	30	..	5–10	5–10			
Blood pressure		160/112	108/40	140/80	110/40	190/140	120/40			
Coma		Early	Early	Early	Early	Early	Early	Late	Early	
Convulsions				Late	Late	..	Yes	..	Late	
Incontinence			Yes	Yes						
Strabismus							Yes	Yes	Yes	
Nystagmus					..			Yes		
Reflexes:										
Deep		0	0	0					0	
Pupillary		+	+	+	0	0	0			
Corneal					+					
Brain lesions:										
Hyperemia	+++	++++	++	++++	++	++	+++	++++	++	+++
Edema	+++	++++	++	++++	++	++	++	++	++	+++
Hemorrhage	+	0	0	+	0	+	+	+	+	+++
Cell degeneration	++	+	+++	++	+++	+	++	++	++	+++
Encephalitis	0	0	0	0	+	0	++++	+++	++	0
Meningitis	0	0	0	0	++	+++	+	0	*	0
Crystals	+	+	*	*	++	+++	++	++	+	+

* Spaces left blank indicate that the item was not mentioned in the clinical record. Items marked * imply a positive finding, but too slight in degree to warrant + designation.
† Temperature not recorded, but patient described as "cold and clammy."

The body temperatures tended to be low, 2 patients having been described as "cold and clammy," and 3 had rectal temperatures of 93° F. One of the latter and another man with a normal temperature had a subsequent rise to 102° F. No readings on the remaining 4 were recorded. The pulse rate ranged from 42 to 120 and the respiration from 5 to 30 per minute, but the two did not always follow the temperature curves. Blood pressure varied from 108/40 to 190/140, the highest being observed in a patient with a temperature of 93° F., pulse 54, and respiration 5. Eight cases presented the clinical appearances of shock.

All but 1 of the men were discovered in deep coma which had supervened at some unknown time within the first 10 seizures toward the end; 3 displayed strabismus; 1 also had vertical and lateral nystagmus. Deep reflexes were absent in the 4 cases tested, although the pupillary reactions could still be elicited. In 3 other cases the pupils were fixed and in 1 only a slight corneal reflex was obtained.

Laboratory Data. The *urine* was examined in 5 cases and invariably contained oxalate crystals; a trace of albumin was present in 4, a heavy cloud in the 5th, in which survival was longest. A few leukocytes were noted in the urinary sediment, but no red blood cells or casts.

A *blood* count was performed in only 1 case, erythrocytes being 7,370,000, hemoglobin 17 gm. and leukocytes 47,900 (86% neutrophils). The non-protein nitrogen level was 47 mg. per 100 cc.* and

* In a case not included in the series because the brain was not examined, the non-protein nitrogen value was 43 mg. per 100 cc.

blood sugar 240 mg., the latter rising to 400 mg. in several hours despite the administration of $\frac{1}{6}$ M. lactate and insulin. This patient was thought to be in diabetic coma until the urine was found to be sugar-free and the CO_2 combining power 46 vol. %. A sample of blood taken 4 hours after death in another case contained 76.9 mg. urea nitrogen per 100 cc.; the validity of this figure is questioned, however.

The *spinal fluid* was described as clear in 3 cases, while in 4 it was blood-tinged. The specimen from the patient with the high blood sugar levels was clear, contained 1 cell per c.mm. and 92 mg. of sugar per 100 cc., and was under a pressure of 160 mm. of water.

Gross Pathologic Findings. The tissues in general were congested and in all cases there was moderate to marked pulmonary edema. The *kidneys* were somewhat swollen and weighed from 200 to 260 gm. in all the cases except the one of longest duration in which each approximated 300 gm. The parenchyma was pallid and prominently striated by engorged vessels, but the markings remained distinct; the pelvic mucosa occasionally showed small patches of hemorrhage. The weight of the *liver* was normal except in 3 instances in which it was recorded as 2100, 2125 and 2350 gm. The organ was occasionally described as soft or flabby, but displayed no distinctive gross change. Injection of the meninges was recorded in 4. Six of the *brains* were prominently hyperemic, 2 of them presenting frank petechiae; 2 others were regarded as grossly edematous with distinctly flattened convolutions. Five weighed between 1500 and 1600 gm., 3 weighed 1400 to 1500 gm.; the other 2 were not weighed. The cerebrospinal fluid was thought to be excessive in 2 cases. The other organs showed no macroscopic lesions of note.

Histopathologic Observations. The gross finding of pulmonary edema was confirmed, with early bronchopneumonia present in 6 cases. Crystals of calcium oxalate* were conspicuous throughout all of the *kidneys* (Figs. 1 and 2), occasionally in glomerular spaces, but for the most part in the renal tubules, sometimes seeming to pierce their walls. Despite this, there was no reaction to their presence in the 9 rapidly fatal cases apart from occasional slight epithelial desquamation. The tubular epithelium did not appear degenerate. The capillary bed was markedly engorged in common with the other tissues. In the kidney of the patient who survived 120 hours there was moderate degeneration of tubular epithelium, especially in the proximal convoluted segments, as well as edema of the interstices; the glomeruli remained apparently normal and there was no cellular reaction. His *liver* weighed 2350 gm. and displayed marked fatty change which was undoubtedly preëxistent; the same condition obtained to a lesser degree in the 2 other enlarged livers. There was evidence of mild parenchymatous degeneration in 2 other instances, but in general the organ was merely congested.

The fine capillary bed of the *brain* in every case was more conspicuous than usual and the larger vessels were engorged. Edema was evident in all to varying degrees, being very pronounced in 2. Perivascular hemorrhage was minor in 6 (Fig. 3 *a*) and prominent in 1 (Fig. 3 *b*). Early acute encephalitis was observed in 2 of the brains (Fig. 4), while in 2 others the inflammatory process was well developed (Figs. 5, 6 and 7). There was an associated meningitis in 3 (Figs. 10 and 12) and a 4th showed pronounced meningitis without encephalitis. The inflammatory exudate was perivascular in the brain and tended to be more diffuse in the leptomeninges. Neutrophils comprised the bulk of the cellular components of the exudate, although there was a distinct histiocytic reaction and an interspersion of lymphocytes.

Unfortunately, the entire brain was not

* The identity of the crystals was confirmed by Mr. William L. Eisenberg, Division of Microbiology, Food and Drug Administration.

available for study in any of the cases, our material being restricted to relatively small blocks of tissue submitted by the laboratory officers who performed the autopsies under the difficulties of field conditions. An evaluation of damage to individual cells in the various regions and nuclei could not consistently be carried out; however, our observations make certain generalizations possible. In the cerebral cortex some of the ganglion cells, especially the Betz cells, displayed moder-

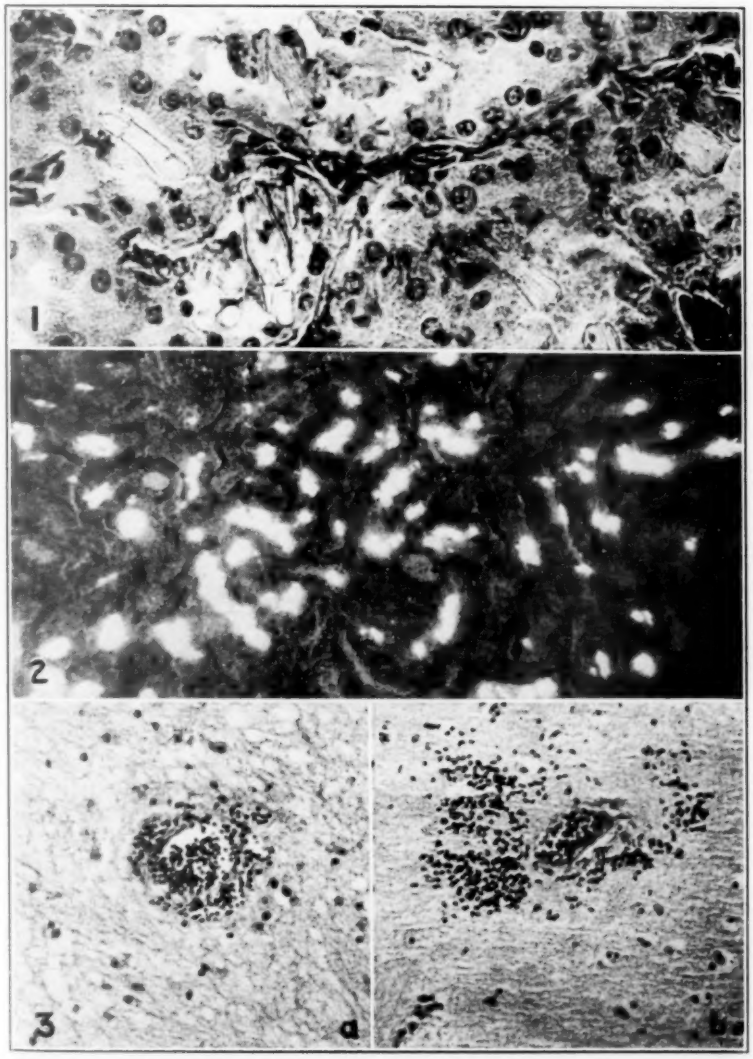

FIG. 1.—Heavy deposit of calcium oxalate crystals in renal tubules, a consistent finding. The uppermost tubule displays a loss of a few lining cells, there being little damage as the patient died within 48 hours. (× 600 slightly reduced.)

FIG. 2.—The same kidney viewed with polarized light shows the wide distribution of the doubly-refractile calcium oxalate crystals. (× 145.)

FIG. 3.—*a*, Early perivascular hemorrhage and marked edema in the white matter of the brain. Survival time, 22 hours. (× 250 slightly reduced.) *b*, Damaged vessel wall and more diffuse hemorrhage. Survival time, 32 hours. (× 250.)

ate chromatolysis and occasional loss of nucleoli and nuclei; satellitosis beyond the normal was sometimes encountered and was most prominent in the case of longest duration. The same alterations were observed in ganglion cells of the brain stem and cerebellum, a few displaying coagulation necrosis, others faded to ghost forms. Perhaps the most striking change was the loss of Purkinje cells of the cerebellum and the evident damage to many of those that remained (Fig. 9).

Histologic examination with *polarized light* served to emphasize the extensive

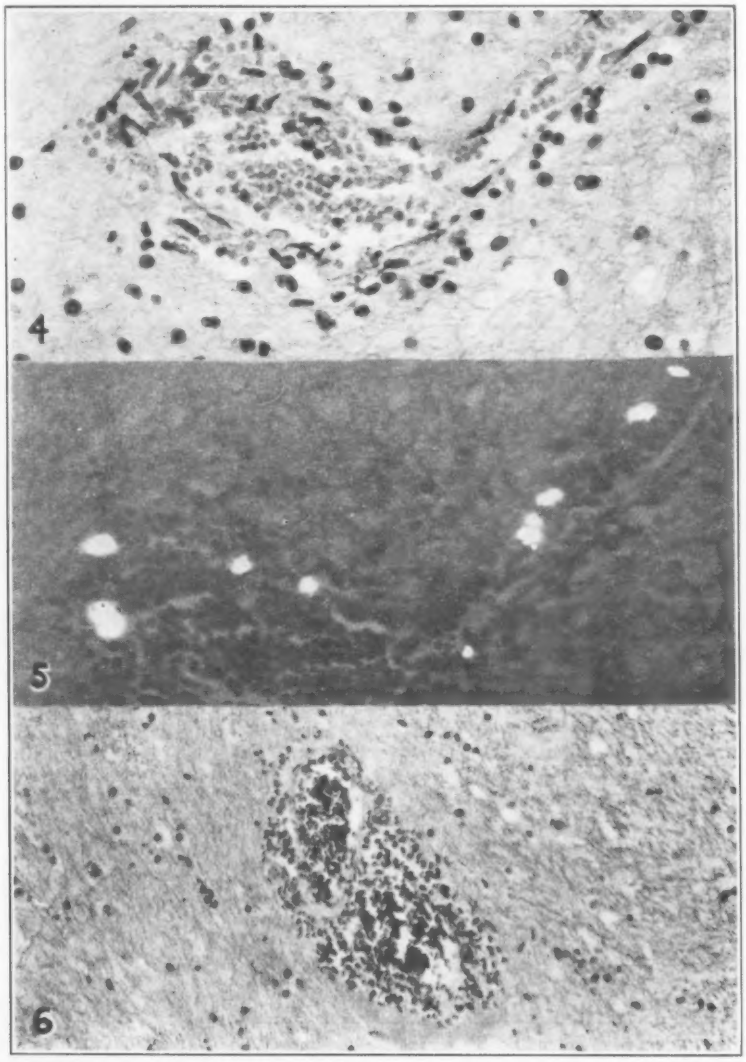

FIG. 4.—Beginning encephalitis, slight perivascular hemorrhage, and marked edema. Survival time, 28 hours. (× 500 slightly reduced.)

FIG. 5.—The same field with polarized light disclosing otherwise invisible crystals, presumably oxalate. (× 500 slightly reduced.)

FIG. 6.—Well-developed encephalitis, the cellular exudate being composed largely of neutrophils. A few crystals were demonstrated with polarized light. Survival time, 43 hours. (× 250 slightly reduced.)

calcium oxalate deposit in the kidneys (Fig. 2), the crystals being doubly refractile. The same means disclosed similar but somewhat smaller crystals in the brain and leptomeninges (Figs. 5, 8 and 11), rather abundant in 4 cases and sparsely distributed in the others. For the most part they lay in the vessel walls and perivascular spaces, sometimes within the vascular lumina. A few crystals were found in the brain substance proper where they evoked no glial or leukocytic reaction. They were clearly in the plane of the sections and could be readily distinguished from doubly-refractile particles occasionally present in the mounting medium employed. Furthermore, they could not be found in a control series

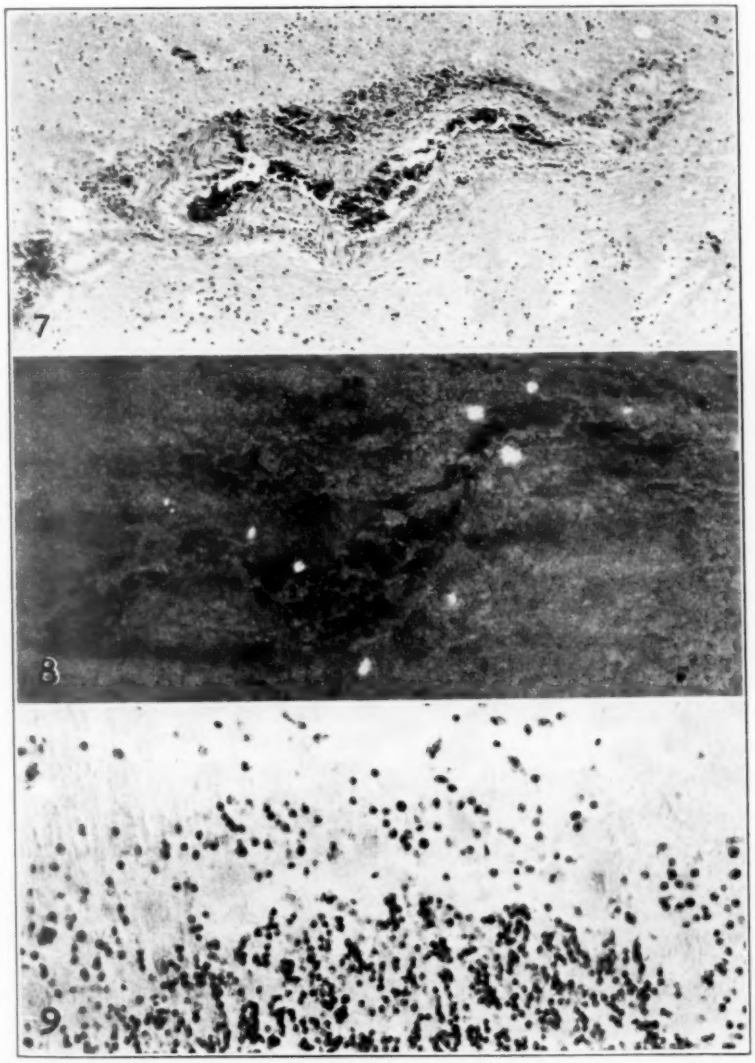

FIG. 7.—Severe encephalitis. Survival time, 41 hours. (× 135.)
FIG. 8.—The same field with polarized light displays deposition of crystals. (× 220.)
FIG. 9.—Cerebellum showing degeneration of Purkinje cells associated with edema and early proliferation in the Bergmann layer. Survival time, 32 hours. (× 300.)

which consisted of brain sections from a group of unrelated conditions. A few isolated crystals were observed in the lungs, but virtually none in other organs.

Discussion. Symptoms referable to the central nervous system were the most conspicuous part of the clinical picture, as has been recognized by others. This is in keeping with the histologic findings in our series, since there were significant intracranial lesions in all 10 cases, which varied from congestion and edema in the mildest to exudative meningo-encephalitis in the most severe.

Crystals, presumably oxalate analogous to the oxalate deposit in the kidneys, were

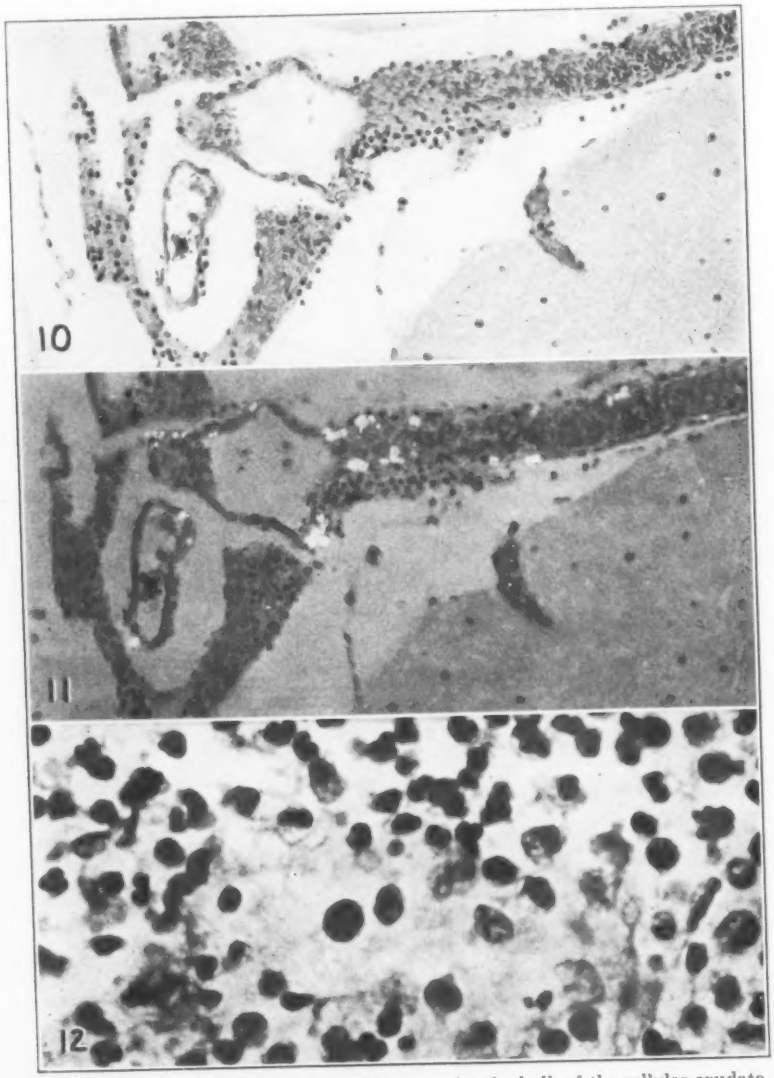

FIG. 10.—Early meningitis in which neutrophils comprise the bulk of the cellular exudate. Survival time, 43 hours. (× 250 slightly reduced.)

FIG. 11.—Polarized light discloses large numbers of crystals in the section shown in Figure 10. (× 250.)

FIG. 12.—Severe meningitis in which there is already an admixture of lymphocytes and histiocytes in the exudate. Survival time, 22 hours. (× 1000 slightly reduced.)

observed in and about engorged vessels of the brain and meninges. It is probable that these crystals are here, as in the kidneys, oxidation products of ethylene glycol formed *in situ*.

The question can be raised as to why these two organs should be virtually the only sites of oxalate deposition. In the kidney, which is the main avenue of excretion of ethylene glycol, it is presumably a matter of concentration. If one may draw an analogy between ethylene glycol and ethyl alcohol as regards absorption, dispersion, and excretion, a possible explanation can be advanced regarding the brain. It has been shown that after administration of alcohol the blood level rises sharply during the 1st hour or 2 and gradually declines, whereas the alcohol concentration of the spinal fluid is built up more slowly but is sustained for a considerably longer time.[8,10] Therefore, in the case of ethylene glycol, it may be of some significance that the crystals were found chiefly where tissue was in contact with cerebrospinal fluid, *i. e.*, in the pia-arachnoid and Virchow-Robin spaces.

Alterations of individual cells in formol-fixed brains are difficult to assess, particularly in older people and in whole brains fixed before slicing. In our series the degenerative changes described are regarded as valid, because all of the patients were young, most of the autopsies were performed soon after death, and small blocks rather than whole brains were placed in the fixative. Furthermore, the fact that some of the degenerating ganglion cells displayed satellitosis supports this view. We believe that damage to the central nervous system is due in part to a direct toxic effect of the chemical and in part to anoxemia resulting from circulatory disturbance, probably also of central origin.

With reference to the kidneys, it appears that if death occurs within 2 days renal damage is minimal, despite the heavy oxalate deposit in the tubules. The single blood urea nitrogen level obtained in this group was high, but was probably not reliable as it was a postmortem sample; urinalyses showed only a trace of albumin. Significant renal changes were found in the patient who lived for 5 days, however, and the urine contained a heavy cloud of albumin.

The more complete data available in 1 case are of interest. There was evidence of hemoconcentration as judged from the blood count and, although the patient presented the clinical appearances of shock, his temperature was 98° F., pulse 96 and blood pressure 160/112. No explanation is offered for this inconsistency. The blood sugar values in this case were much higher than one can attribute to hemoconcentration and, whereas propylene glycol is converted to glycogen in the animal body, ethylene glycol is not. Glycogen may have been released as a result of liver damage, hardly apparent on histologic examination, or it is possible that ethylene glycol or some product of its intermediate metabolism may have acted as a reducing agent in the test for sugar.

Since this paper was prepared 8 additional cases of acute ethylene glycol poisoning have been observed. The clinical and pathologic findings were identical with those described in the 10 original cases.

Conclusions. Acute ethylene glycol poisoning produces demonstrable lesions of the central nervous system and by this means is responsible for death, as shown in each of 10 fatal cases reported here, and 8 more mentioned in the addendum.

Renal damage severe enough to contribute to a fatal outcome was found in only 1 patient who survived for 5 days; in the other cases there was nothing beyond simple deposition of calcium oxalate.

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SUCCESSFUL COMBINED TREATMENT OF PENICILLIN-RESISTANT GONORRHEA

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Recent articles have reported spectacular success in the treatment of gonococcic infection with penicillin. In May 1943 Herrell, Cook and Thompson[5] reported the results of the use of penicillin in the treatment of 3 cases of gonorrhea. Since that time many publications have appeared, particularly those of Keefer and his associates,[6] Mahoney and others,[7] Ferguson and Buckholtz,[4] and Sternberg and Turner,[8] which have shown that gonorrheal infection responded very rapidly to penicillin treatment. All these observers achieved gratifying results in the treatment of male patients. Cohn, Studdiford and Grunstein[1] studied 44 female gonorrheal patients of whom a total of 43 promptly became bacteriologically negative after treatment with penicillin and remained negative during the follow-up period. In the treatment of a large series of cases the efficacy of penicillin in the sulfonamide-resistant gonococcic infections has been almost complete. Sparse comments have been made on those cases which failed to respond to the usual penicillin treatment. We consider in this report 4 cases which failed to respond to adequate penicillin therapy.

Report of Cases. Case 1. A married American white soldier, aged 27, was seen on March 20, 1945. He had gonorrhea in 1939 which was adequately and satisfactorily treated. He was sexually exposed on Nov. 2, 1944. He was admitted to hospital at Camp Swift, Texas, on Nov. 13, 1945, with a urethral discharge, smear of which was positive for gonococci. A total of 34 gm. of sulfathiazole was administered in 5 days. This treatment was discontinued on November 17. The next day the urethral smear was still positive for gonococci, in spite of the fact that, in addition to sulfathiazole, penicillin had also been started on November 15. The patient was given 200,000 units of penicillin intramuscularly on this date. On November 16, 400,000 units were given. Additional penicillin was given on November 18, 19, 20 and 21. A total of 2,600,000 units was administered in this course. Repeated urethral smears continued to show gonococci. Because of this, the patient again was placed on penicillin therapy. From November 27 to 30, 1,600,000 units were given. Urethral smear examinations done on November 27, 28, 29, 30 and 31 were positive for gonococci. On December 9, argyrol instillations were begun and continued until December 22. On December 22, penicillin was again started and continued for a period of 3 days. Argyrol instillation was again given for a period of 3 days following penicillin therapy. This patient was returned to duty on Dec. 28, 1944. Urethral smears performed on December 25, 26 and 27 were negative for gonococci, but his discharge persisted until March 1, 1945, at which time he developed a profuse recurrence. The patient denied any sexual intercourse since Nov. 10, 1944. On March 5, 1945, he was admitted to the Regional Hospital. Repeated urethral smears were positive for gonococci. On March 20 he was transferred to Lawson General Hospital as a sulfonamide and penicillin-resistant case of gonorrhea. Here also positive urethral smears were obtained. Urethral cultures were also positive. Fermentation tests confirmed the presence of the gonococcus. Penicillin assay study *in vitro* failed to show any inhibition. On March 27, April 5 and April 14 he was given fever therapy. After the third fever session the urethral discharge disappeared. Repeated urethral smears and cultures thereafter were negative.

Case 2. This 23 year old white soldier was inducted into the army in November 1942. He was transferred from Fort Jackson, S. C., Jan. 23, 1945, for the treatment of resistant gonorrhea. This patient stated

that he had sexual intercourse on Oct. 28, 1944. He noticed an acute urethral discharge 8 days following intercourse. A diagnosis of gonorrhea was made Nov. 10, 1944. Repeated urethral smears were positive. On November 10 he was given 100,000 units of penicillin intramuscularly. Urethral smears continued positive. On November 18 he was given 200,000 units of penicillin. This failed to check the discharge and the positive smears. On November 27 he was hospitalized and given 300,000 units of penicillin. Two consecutive urethral smears were negative for gonococcus. He was returned to duty on Dec. 1, 1944. Follow-up examinations were again positive for gonococcus. On December 13 the patient was again hospitalized for urethral discharge. Sulfadiazine was started on December 15 and continued to December 21. A total of 32 gm. was given. The discharge persisted; the urethral smears continued positive for the gonococcus. Follow-up urethral smears continued positive until he was transferred to Lawson General Hospital for fever therapy. Examination at this hospital continued to show a heavy purulent urethral discharge with repeated positive urethral smears and cultures. Identification of the gonococcus was confirmed by sugar studies. During his stay at this hospital he was given 32 gm. of sulfadiazine without any clinical or laboratory improvement. Penicillin assay was performed. No evidence of inhibition *in vitro* was shown.

Case 3. This 25 year old white soldier was inducted Feb. 24, 1941. He states that early in November 1944 he had illicit sexual intercourse. On Nov. 4, 1944, he noticed a urethral discharge. He was given 100,000 units of penicillin while on duty status. Repeated urethral follow-up smears were positive for gonococci. On Nov. 18, 1945, he was given an additional 200,000 units of penicillin. Urethral smears continued positive. It was necessary to give the patient more treatment. On November 24 he was given 200,000 units of penicillin. Urethral smears were still positive. A heavy urethral discharge persisted. 300,000 units of penicillin were administered on November 28. Follow-up studies demonstrated positive smears. On December 28 sulfadiazine was instituted. A total of 40 gm. were given. The urethral discharge persisted. The smears were positive. The smears continued positive until Jan. 13, 1945. Because this patient failed to respond to sulfadiazine and penicillin he was transferred to Lawson General Hospital on Jan. 24, 1945. Numerous urethral smears and cultures were found positive for gonococci. Sugar studies were done to confirm the identification of the organism. Penicillin assay *in vitro* was carried out. No inhibition was shown by the cup method used. This patient was given fever therapy and additional 480,000 units of penicillin at the height of the fever. Two sessions of fever were necessary to cure this patient.

Case 4. This 22 year old colored soldier was inducted July 13, 1944. This patient states that at the time of his induction he had a urethral discharge. According to his story, he first had gonorrhea in 1936 which failed to be completely cured until 1940. During this time he received many forms of treatment. His discharge did not recur until June 1944 prior to his induction. This he believes to be a new gonorrhea, since he had had frequent illicit sexual intercourse. This patient's urethral discharge continued until Aug. 9, 1944, when he was hospitalized and treated for acute gonorrhea. During this hospitalization he was given sulfathiazole, sulfadiazine and a total of 600,000 units of penicillin. The urethral discharge and positive smear continued. On September 28 he again was hospitalized for fracture of the left leg. While receiving treatment for his fractured leg his urethral discharge persisted. On November 6 he was transferred to the venereal ward for further treatment of his gonorrhea. He was given 100,000 units of penicillin. The urethral discharge persisted. The smears continued positive. Beginning on Nov. 17, 1944, he was given 400,000 units of penicillin; no improvement noted. Laboratory studies repeated on numerous occasions were found to be positive for gonococci. This patient was transferred to Lawson General Hospital on Jan. 20, 1945, as a case of penicillin-resistant gonorrhea. The urethral discharge was present and profuse. Repeated urethral smears and cultures were positive for gonococci. Sugar reactions confirmed the identity of the organism. Penicillin assay was performed. This confirmed the clinical findings. No inhibition was demonstrated. Two fever sessions were necessary to cure this case.

Comment. In recent literature thousands of cases of gonorrhea have been treated without encountering instances of penicillin resistance. Sternberg and Turner[8] in a series of 1686 cases failed to observe any cases of penicillin-resistant gonorrhea. This observation was confirmed *in vitro* by Cohn and Seijo[2] in which they showed that a 1–10,000 dilution of penicillin killed all the gonococcic strains tested. At this hospital 4 cases were studied by the penicillin assay method. These cases had positive urethral smears and cultures. Fermentation reactions were used to prove the identity of the gonococcus. Penicillin assays showed that the drug had no effect on the growth of gonococci. No inhibition to growth was shown. The method of titration of *N. gonococci* with penicillin (modification of the Oxford method) was as follows: (1) Chocolate blood agar plates containing approximately 25 cc. of agar are seeded with 0.5 cc. of a 24 hour broth culture of *N. gonococci* and allowed to dry for ½ hour. (2) With a sterile glass cylinder a hole was cut in the center of each plate. The bottom of the hole was then sealed with 2 drops of nutrient agar. (3) This cup was filled with 2 drops of penicillin, each drop containing approximately 400 units of the drug. (4) The plates were incubated in CO_2 at 37° C. for 24 and 48 hours. Examinations in all 4 cases failed to show any zone of inhibition. What factors influence the lack of inhibition in these cases have not been determined. Yet, from clinical and laboratory studies, penicillin failed to cure these patients. Wright[8] in his discussion of Sternberg and Turner's paper indicated that some patients continued to have a profuse urethral discharge with positive cultures for 6 months or more. The possibility that the excretion of the penicillin in the discharge was a factor in inhibiting the culture growth was ascertained. Two of Wright's patients, both men, had had a profuse discharge for months with positive smears. Yet cultures were negative. Repeated sulfonamide, penicillin and fever treatments failed to alter the discharge or the positive urethral smear findings. A failure rate of 5.2% with penicillin was reported by Dunfield and Mandel[5] in a series of 154 cases of uncomplicated gonorrhea. This was determined by the presence of discharge, positive urethral smears and cultures.

Summary. Four cases of penicillin-resistant gonorrhea were reviewed. All cases failed to respond to large doses of penicillin. Gonorrhea was proven by urethral smears and cultures. Fermentation reactions were also used. The organisms were shown to be penicillin resistant by *in vitro* studies. Combined sulfonamide-penicillin and/or fever treatments were employed in all of the 4 cases of resistant gonorrhea. All of the cases responded to this form of treatment. Penicillin assays employed by the usual bacteriologic method in 4 cases at this hospital indicate that penicillin-resistant strains of gonococci do exist.

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MENINGOCOCCUS ENDOCARDITIS

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Although originally described by Weichelsbaum[34] in 1887 as the etiologic agent of a specific type of meningitis, the Meningococcus (*Neisseria intracellularis*) is now known to be the etiologic agent not only of one type of meningitis, but also of a generalized septicemia which may be followed by bacterial localization in many different organs, producing a number of clinically distinct and characteristic syndromes. One of these, meningococcic endocarditis, has reached recent prominence as a disease process which may be susceptible to the newer therapeutic agents. However, the fulminating nature of the disease, combined with the difficulties in establishing an early bacteriologic diagnosis, often conspire to delay therapy until the patient's condition is beyond the point of reversibility.

Because of the relative infrequency of primary meningococcic endocarditis, a case will be described in detail, and the natural history of the disease, as derived from 24 cases collected from the available literature, will be discussed.

Case Report. W. B., a 23 year old married gasoline station attendant, entered the Salt Lake General Hospital on January 10, 1945 complaining of weakness, dry cough, weight loss and "heart trouble." The patient had been perfectly well until November 18, 1944, when he developed a severe sore throat and fever. He noticed that lateral, but not anterior-posterior movements of the head produced pain. He went to bed where he remained for 4 to 5 days. The sore throat disappeared, but general aches and pains and malaise supervened, making it impossible for the patient to return to work.

Two weeks after the onset of his illness, he consulted a physician who, after a careful examination, told him he had "bad tonsils" but that he was otherwise normal. He returned home, but because of continued malaise, anorexia, and weight loss, he again consulted his physician 2 weeks later. On this occasion, a low grade fever and a cardiac murmur were found. Because of his continued down-hill course, the patient was told to apply to the hospital for admission. He had observed no rash. There had been no arthralgia, petechiæ, or symptoms referable to embolic phenomena. He complained only of a slight dry cough. He had had no stiff neck since the day of onset, and no other symptoms or signs of meningitis. There had been a loss of weight from 158 to 139 pounds since the onset of his illness.

The *past history* revealed that he had had diphtheria at the age of 7 years, but there was no history suggestive of rheumatic infection or impairment of cardiac reserve. He denied any venereal disease and previous blood serology tests for syphilis had been negative. At army induction examinations in February and again in May, 1944, no cardiac disturbance was noted.

The admission *examination* revealed an acutely and chronically ill, pallid young man who appeared extremely weak but who was in no acute distress. A temperature of 101.6° F. and a pulse rate of 120 per minute were found on admission; the respiratory rate was 28 per minute and the blood pressure 135/20. Except for "freckles" on the shoulders, the skin was negative. Examination of the head and neck revealed no abnormality other than approximately 8 to 10 pea-sized lymph nodes in the anterior and posterior cervical chains bilaterally. The thorax was negative except for the heart, which was enlarged 2 cm. to the right of the midline. The left border of cardiac dullness was percussed at the left mid-clavicular line, and there appeared to be some straightening of this border. A tachycardia was present, but the rhythm was regular. The tones were vigorous, and the precordium heaved moderately. There were no thrills. A loud harsh systolic murmur, heard best at the aortic area, was transmitted to the neck vessels and subclavian arteries bilaterally. A loud, high pitched, early diastolic murmur was heard along the left sternal border. The first and second aortic

sounds were heard clearly. A reduplicated second sound was present at the mitral area, and a loud harsh systolic murmur accompanied by a low pitched diastolic rumble, differing in pitch and intensity from those at the base, were heard at the apex. A Corrigan pulse could be felt in all the peripheral arteries, and Duroziez' sign, as well as capillary pulsation of the nail beds, were also present. Examination of the abdomen was negative, except for a questionably palpable spleen. The remainder of the physical examination was negative.

Blood examination disclosed: erythrocytes, 4.2 million cells per cmm.; hemoglobin, 12.5 gm. %; volume of packed red cells, 32 cc. %; icterus index, 2 units; corrected sedimentation rate (Wintrobe) 35 mm. The total leukocyte count varied from 8000 to 13,400 cells per cmm., and the differential count revealed 6 to 18% juvenile granulocytes, 60 to 70% polymorphonuclears, 0 to 5% eosinophils, 0 to 1% basophils, 12 to 17% lymphocytes and 2 to 8% monocytes.

Urine examinations were negative except for 1+ albuminuria on 1 of 6 determinations, and "occasional" red and white blood cells. A non-hemolytic staphylococcus, and a pneumococcus were cultured from the throat. Urine cultures and prostatic smears and cultures were negative. Three of 6 venous blood cultures revealed a gram-negative diplococcus. One arterial blood culture was negative, but one culture of sternal marrow yielded a better growth of the same gram negative organism than did the cultures of venous blood. It is noteworthy that these cultures did not become positive until 7 to 9 days after the blood had been drawn. Because these organisms fermented maltose and dextrose with the production of acid, but did not ferment levulose, they were classified with the *N. intracellularis* group. No typing sera were available.

Roentgenograms of the chest revealed nothing of note. The heart size and contour were normal. The transverse cardiac diameter was 13.3 cm. and the transverse intrathoracic diameter was 31 cm. The electrocardiogram revealed a heart rate of 100 beats per minute with a P–R interval of 0.16 sec. and QRS of 0.08 sec. T wave inversion was noted in Lead I on admission. This became more marked during the course of the illness. Serial chest leads were in no way remarkable except as they demonstrated inverted T waves over the left ventricle.

The hospital *course* of this patient was, at first, uneventful except for the continuous spiking fever which was often of the "double quotidian type" (see Fig. 1). Weakness and debility increased progressively. On January 23, 1945, 15 days after admission, the first positive blood culture was reported and penicillin was begun. An initial dose of 20,000 units was given intravenously and this was followed by 20,000 units intramuscularly every 3 hours.

During the evening of the same day, the patient complained of general abdominal pain and inability to void. On the following morning, catheterization was performed, but the bladder was empty. Abdominal distress was marked and an indefinite tender mass was palpable in the right upper quadrant. The abdomen was distended with gas and bubbling râles could be heard in the posterior chest. For the first time, ecchymoses appeared on the right foot. Within a few hours, the liver was palpable 5 finger breadths below the costal margin, and numerous loud coarse râles could be heard throughout the chest. The venous pressure was 250 mm. of water. The blood urea nitrogen at this time was found to be 34.7 mg. per 100 cc.; the plasma chloride, 433 mg., and the CO_2 combining power 19 vol. %. The electrocardiogram revealed a P–R interval of 0.16 sec. with widening of the QRS complex to 0.16 sec. The heart rate at this time was 110 beats per minute. Serial precordial leads demonstrated changes compatible with a uniform depression of myocardial conductivity. Despite sedation, parenteral ouabain, aminophylline, bloodless phlebotomy, and nasal oxygen, the patient failed rapidly and died within a few hours.

AUTOPSY (2 hours after death) yielded the following positive findings on gross and histologic examination:

Gross examination of the *heart* revealed a normal epicardium and myocardium. There was a massive fresh thrombo-ulcerative endocarditis of the left anterior aortic valve cusp, which had ulcerated through the septum and protruded into the left atrium as a mural thrombus just above the mitral valve. This latter valve showed only a minimal vegetative process on the medial cusp. There was no evidence of pre-existing valvular deformities. On histologic examination, the myo-

cardium showed moderately advanced parenchymatous degeneration and areas of leukocytic infiltration suggesting acute focal myocarditis. No Aschoff bodies were found.

Multiple small and large hemorrhages were noted throughout the *peritoneum*. There was a fibrinopurulent peritonitis, and bilateral hydrothorax, hydropericardium, ascites and anasarca. The *kidneys* showed healed infarcts of various sizes and ages, parenchymatous degeneration and passive congestion. Focal (embolic) subacute glomerulonephritis was also present. There was congestion of the *liver* and congestion and edema of the *lungs*. The *spleen* weighed 450 gm. and showed anemic infarcts and diffuse hyperplasia.

Permission was not granted for examination of the central nervous system.

Incidence. The incidence of meningococcic endocarditis, as compared with other types of endocarditis, appears extremely low in most statistical studies. Thayer[32] described 198 cases of acute bacterial endocarditis in Baltimore, Williams[38] 38 cases in Nashville, Lenhartz[21] in Germany and Horder[14] in England, 134 additional cases, and Clawson[4] collected 46 cases—a total of 416 cases all together in which bacteriologic studies were done. None of these instances of endocarditis were attributed to the meningococcus. The gonococcus was described in 2 to 12% of cases in the various series. Harbitz[9] found that 2 out of 32 of his cases of acute bacterial endocarditis were due to a "gram negative" diplococcus, but he did not further identify the organism. Dauphinee[6] reviewing 304 cases of endocarditis, stated that the meningococcus is "only rarely" the etiologic agent.

However, White[37] stated that in a group of 48 cases of acute bacterial endocarditis in Boston, 4 were caused by the meningococcus and an equal number by the gonococcus. Because of this study and also because of the comparative frequency of recent isolated reports of meningococcic endocarditis in the literature, we suspect that the incidence of primary acute bacterial endocarditis caused by the meningococcus is higher than has been hitherto found. There is evidence to suggest that the earlier authors did not differentiate clearly between the gonococcus and the meningococcus, which may account for the scarcity of reports of meningococcic endocarditis in the older literature.

Herrick[13] was the first to describe the usual course of meningococcic sepsis, which he found to occur in 3 stages. The first, lasting 2 days to 6 weeks, is a local inflammation of the upper respiratory passages. This is followed by a bacteremia lasting approximately 48 hours, after which the bacteria localize in the meninges, the heart, or at other sites throughout the body. This "average" picture is subject, however, to infinite variation in length and order of stages, or the disease may terminate at any stage without progressing further.

Thus we find that endocarditis can occur as a complication of meningitis, as a complication of meningococcic septicemia, or as a "primary" disease entity. Endocarditis was found to be a complication of meningococcic meningitis in 6% of Smithburn's cases,[29] but was not mentioned as a complication in a series of 4464 cases reported by Horowitz and Perroni.[16] In the Army during the first World War, endocarditis was found by Krumbhaar and Cloud[19] in 3 cases all of which also showed signs of meningitis. Approximately half of the cases of meningococcic endocarditis have followed meningitis.[22] In 12 to 27% of cases of recognized chronic meningococcic septicemia endocarditis has developed.[1,2,26] It is difficult to determine accurately the proportion of cases of endocarditis that are preceded by a simple, comparatively long-standing meningococcic septicemia, although a history suggestive of preceding septicemia of 2 or more weeks' duration was found in 5 of 11 of this series.

Pathology and Pathogenesis. In contradistinction to the more familiar streptococcus viridans endocarditis, meningococcic endocarditis usually develops upon previously undamaged heart valves. In only 3 of 19 autopsied cases reported in the literature cited here was there evidence of old chronic valvular disease in addition to the acute valvulitis. In another report[17]

no mention is made of the possible presence of luetic aortitis in addition to the acute aortic valvulitis, although the patient's Wassermann was recorded as 3 plus. In an additional 7 cases that were not autopsied, there was no history of previous rheumatic fever or heart disease.

Like *Streptococcus viridans* endocarditis, meningococcic endocarditis usually involves the left side of the heart. Of 26 autopsied cases, the mitral valve alone was involved in 17, the aortic in 3, both aortic and mitral in 5, and aortic and tricuspid in one. Pericarditis was also described in 3 and myocarditis in 9 out of 18 of these cases. Meningococcic myocarditis, according to Saphir,[28] is characterized by a hemorrhagic exudate, and destruction of muscle fibers, accompanied by the early appearance of endothelial leukocytes and the finding of gram negative intracellular diplococci. In later stages, foci of necrosis, such as are found in subacute *Strep. viridans* endocarditis, appear.

The pathogenesis of meningococcic endocarditis in previously undamaged hearts, has been studied by Miller[25] in horses. In these animals, he described an initial endothelial edema which gave rise to a separation of the endothelial layer from the subendothelial tissue. This produced a wrinkling, fragmentation, and desquamation of the endothelium. Gram negative diplococci were found on the surface and within these desquamating areas. On some valves, fibrin layers containing endothelial cells, blood elements and bacteria, and on others early reparative growths of fibroblasts and endothelial buds could be seen. These changes progressed through stages leading to that also described in human autopsy material, where the typical finding is that if a friable thrombotic vegetative process protruding from the surface of a valve that also shows acute ulcerative and productive inflammatory changes.

Miller[25] suggested that these early endothelial changes may be the result of an "alteration in the course of immunization" which produces an endothelium "more vulnerable to the toxic products" of the meningococcus. These toxic products, then, prepare the way for actual colonization on the valve surface. He showed that the bacteria probably do not enter through end-arteries in the valve, but rather, localize on the surface of the damaged endothelium. His theory of a primary toxic endothelial damage was supported by the finding of similar endothelial changes in arteries of the lung and other organs.

Clinical Picture. In 24 cases, 17 were males and 6 females. Except for 1 Negro all of the cases were in the white race. One patient was 13 years old, 7 ranged between the ages of 15 and 25, 6 between 25 and 35, 4 between 35 and 45, and 3 were over 45 years of age.

Three modes of onset of primary meningococcic endocarditis can be distinguished. Infrequently (2 of this series) the patient, previously perfectly well, is overcome with symptoms and signs of acute heart failure: dyspnea, orthopnea, cough productive of pink foamy sputum, dullness and bubbling râles in the chest, a dilated heart with rapid pulse and heaving precordium, a large tender liver, peripheral edema and cyanosis.

In other cases (11 of this series) the onset is that of an acute septicemia with chills, high temperature and marked prostration. Finally, as in 11 of this series, the onset may be subacute. In these, the patient characteristically believes that he has "the flu," but instead of recovering rapidly, his anorexia, malaise, fatigue, and weakness continue and progress until within weeks or months he appears before the physician, a chronically ill and prostrated individual. A 4th mode of onset of endocarditis, in which the cardiac involvement follows a definite meningococcic meningitis, will not be discussed here since the diagnostic problem can be quickly solved by careful neurologic examination together with a microscopic examination of the spinal fluid.

Certain symptoms and signs are of clincal significance in the diagnosis of meningococcic endocarditis, but do not dis-

tinguish it from simple meningococcic septicemia (Fig. 1).

The arthralgia, which was present in half the cases of this series, is characterized by its mildness. Joints most often involved are knees, ankles, elbows and wrists. Usually pain and tenderness alone are present, but occasionally objective findings of acute arthritis—heat, swelling, redness and fluctuation—are found in some degree. Roentgenographic evidence of joint changes has not been described. This arthralgia is usually present for only a few days or weeks in the early course of the disease and does not recur.

The rash, which occurred in approximately half of these cases, is extremely variable in its appearance. Occasionally a macular rash is present that resembles the rose spots of typhoid fever. The rash may be papular or pustular in character. On occasion it has been compared to that of secondary syphilis and in other cases it has resembled erythemia multiforme or erythema nodosum. In many cases, a petechial or purpuric eruption is present

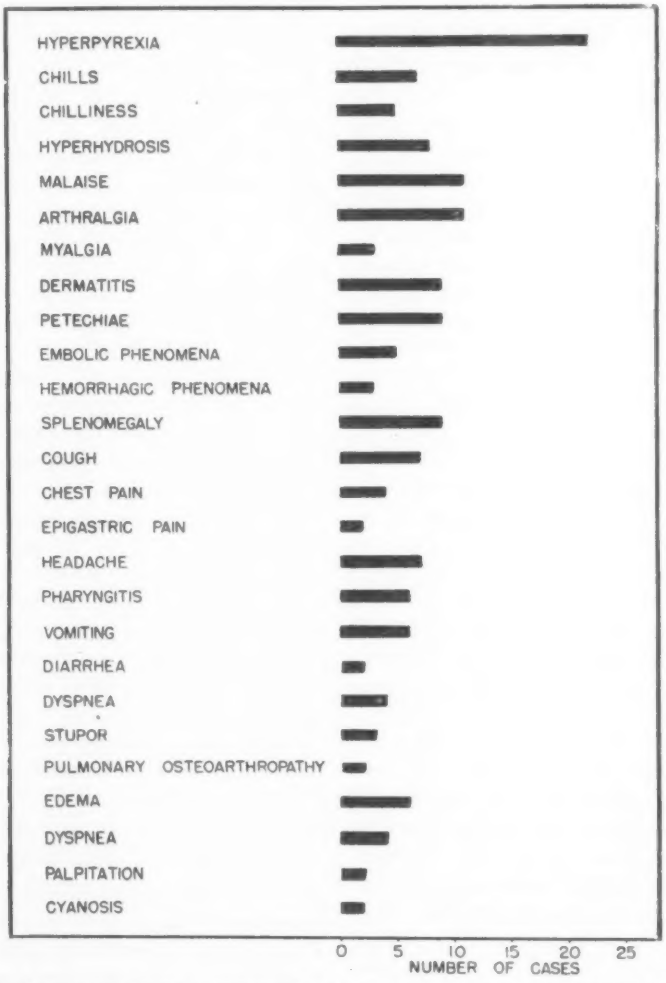

FIG. 1.—The frequency of certain symptoms and signs in 22 cases of meningococcic endocarditis.

alone or in addition to one of the other forms. The duration and extent of the rash are as variable as the morphology itself, and it may occur at any time during the course of the disease.

Other commonly found symptoms and signs are malaise, anorexia, headache, nausea and vomiting, muscular aches and pains, chilliness or true chills, night sweats, cough and pharyngitis, such as one might expect in any severe bacterial systemic disease. The spleen was palpable in ⅓ of the cases in this series.

Another set of symptoms and signs, no one of which was present in more than 20% of this series before the terminal stage, suggested cardiac involvement. These included embolic phenomena (other than petechiæ), chest pain, osteoarthropathy, Osler's nodes, palpitation, cyanosis, peripheral edema, dyspnea and cardiac dilatation.

Hennell[12] and Carbonell[2] have commented on the frequency with which a diagnosis of malaria has been made in these cases because of this type of temperature curve. Often the same patient may show different types of temperature curves during different stages of the disease.

Our case demonstrated a 4th type of temperature curve (Fig. 2) which has not previously been described in meningococcic infections, although it can be seen in the temperature graph of the patient reported by Willius[39] (Fig. 3). This type of fever has been described in gonococcus endocarditis by Horder and Gow,[14] by Williams[38] and under the title of the "double quotidian" fever of gonococcus endocarditis by Futcher.[7] As the name would imply, this fever is characterized by two distinct peaks occurring each day in the temperature curve. Our patient demonstrated this phenomenon on 6 of the 15

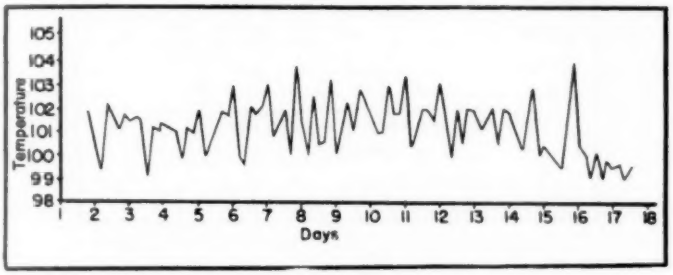

FIG. 2.—Temperature chart of Patient W. B.

In addition, the usual physical signs of involvement of the specific valves were present in all but 1 case at some time during the course of the disease.

Many different types of febrile reaction have been described in meningococcic endocarditis. Occasionally there is a chronic low-grade fever that undulates up and down at irregular intervals. Usually it is a definite spiking type of curve with elevations to 103 to 105° F. each evening, and subsiding to normal by the following morning. Frequently a normal or slightly elevated temperature will be interrupted every 2d, 3d or 4th evening by chills and spiking temperature to 103 to 105°. Both

days during which he was observed. It can no longer be said, then, that this type of fever is characteristic only of endocarditis due to *N. gonorrheæ*. The clinician is, therefore, advised, when he encounters this "double quotidian" fever in a case of endocarditis, to search diligently for both *N. gonorrheæ* and *N. intracellularis*.

The laboratory findings in cases of meningococcic endocarditis are similar to those encountered in any of the bacterial endocardidities. Erythrocyte count, hemoglobin, and hematocrit, show a variation from the normal to a profound anemia which is usually of the normocytic type. Some degree of anemia was found in 12 of 16

cases in this series, and the anemia usually increased with the severity and prolongation of the disease.

The leukocyte count varied from normal values to counts as high as 28,000 per cmm., with a differential often, but not always, showing a marked "shift to the left." In the cases cited in this report leukocytosis was recorded in 17 of 22 cases during some part of the illness. As in other severe infections the more toxic cases in this series often showed a marked "shift to the left" in the differential count without any increase in the total number of leukocytes. The erythrocyte sedimentation rate is usually elevated in this disease. In some cases, albuminuria and microscopic hematuria and pyuria have been recorded. These are usually slight and result, apparently, from renal infarction or from focal embolic nephritis.

Roentgenograms of the heart reveal changes due to dilatation of the various chambers, and the electrocardiogram often records changes in pattern of a non-specific type.

Blood cultures were positive for the meningococcus in all 24 cases cited. It must be emphasized that this organism can be grown only with difficulty and on enriched media such as blood or chocolate agar and in an atmosphere of increased carbon dioxide tension. Since it is very sensitive to light, cold, and desiccation, material for culture should be kept warm and cultured as soon as possible and in generous amounts on warm moist slants of the enriched media. Although venous blood is usually adequate for culture, we have found, as was suggested by Horowitz and Perroni[16] that cultures of sternal marrow appear to yield more colonies per volume than are obtained from simultaneous venous puncture. One arterial blood culture was of no greater value than venous blood, in our hands.

Differential Diagnosis. Because of the almost universal presence of a significant cardiac murmur in cases of acute endocarditis, it is usually not difficult to distinguish disease of this category from cases falling in the vast group of "fever of unknown origin." The clinical picture of meningococcic endocarditis is not sufficiently clear cut so that it can be distinguished with certainty from the other varieties of acute endocarditis. However, there are 3 characteristic signs and symptoms which, when present, make one suspicious of this diagnosis, and which tend to rule out other etiologic agents. These are the presence of skin lesions, arthralgia, and a tertian, quartan, or double quotidian temperature curve. The absence of previous valvular damage and the absence of pneumonia, abscess or other local infections tends to rule out certain other types of acute endocarditis.

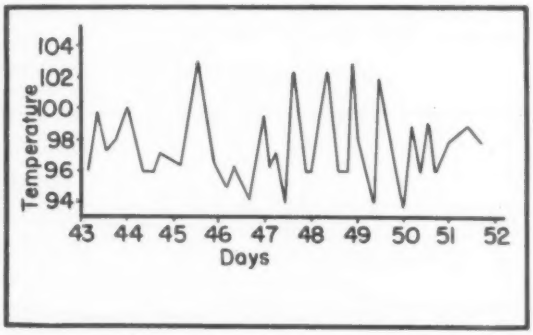

FIG. 3.—Temperature chart reported by Willius.[39]

Endocarditis due to the pneumococcus in the preponderant majority of cases is a complication of pneumococcic pneumonia or some other local pneumococcic infec-

tion.[32,33] Arthralgia and skin lesions, other than those due to petechiæ, are rarely encountered, and the disease is usually more fulminating than that caused by the meningococcus.

In staphylococcic endocarditis, Thayer[32] found pre-existing valvular disease in approximately half the cases, in contrast to the pre-existence of valvular disease in only 13% of the cases due to the meningococcus reported in this series. In $\frac{2}{3}$ of the cases the endocarditis was a complication of a previously recognized staphylococcic infection and usually pyemia was present. Arthritis was found in 24% of the patients, but none had any rash other than petechiæ.

Gonorrheal endocarditis presents the most difficult differential diagnosis, since in most cases no portal of entry of the bacteria is evident, since about $\frac{1}{3}$ of cases demonstrate an arthritis, and since the course and temperature curves resemble those of meningococcic endocarditis very closely.[32] The statistically greater incidence of this disease in the colored race and the usual absence of any true skin rash, are perhaps the only methods of distinguishing the 2 diseases other than by bacteriologic studies.

In acute endocarditis due to a streptococcus (usually β-hemolytic streptococcus[32]) a definite portal of entry was found in about 60% of cases. About $\frac{1}{4}$ of the patients complained of arthritis, but none had a significant rash. About 60% of these acute cases represented infection superimposed on previously damaged valves.

Course, Prognosis and Treatment. The course of meningococcic endocarditis is usually acute and may be fulminating. The duration in 16 of 21 cases in this group was less than 9 weeks. The shortest course was 10 days and the longest over 8 months. Unless specific treatment is instituted, the patient fails steadily until death ensues from the toxemia, from heart failure, or from embolic phenomena.

Most authors state that the prognosis is very poor; but Swift[31] finds that the outlook in this disease is better than in most bacterial endocardidities. In the series reported here, recoveries were reported in 5[5,20,23] of 23 cases, although in 2 of these the diagnosis may be questioned, and in none was the follow-up sufficiently long to rule out recurrences. Antiserum alone was given in 3 of these recovered cases;[23] antiserum, hyperthermia, and prontosil were used in the 4th;[20] and sulfanilamide, sulfapyridine, and sulfathiazole in the 5th.[5] Other authors have used all combinations of antiserum, transfusions, sulfonamides, hyperthermia and penicillin, with no remarkable results. Penicillin, has, thus far, not been found to be as effective against other infections due to the meningococcus as are the sulfonamides, but because of the serious nature of endocarditis, a full combined therapeutic course of penicillin, sulfonamide, and antisera should probably be given in all cases of this disease. Anticoagulants have not been tried in any cases of this series. Their value in the treatment of other types of endocarditis is still in dispute.[18]

Summary. One hitherto unreported case of primary meningococcic endocarditis, together with 24 others collected from the literature, have been presented. It is stated that in many cases the clinical picture is quite characteristic of this bacteriologically specific type of acute endocarditis; so much so that one may often obtain, in individual cases, a definite clue as to the etiologic diagnosis. Most characteristic in the clinical picture of meningococcic endocarditis, are the presence of skin lesions, arthralgia, and a tertian, quartan or double quotidian type of temperature curve, together with the symptoms and signs of acute septicemia and physical signs of cardiac valvular involvement.

Dr. Hans H. Hecht gave advice and criticism during the preparation of this study.

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AZOTEMIA IN GASTRO-INTESTINAL BLEEDING

THE INGESTION OF SHED BLOOD IN HUMANS

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THE past decade has seen numerous workers drawn to the problem of azotemia resulting from gastro-intestinal hemorrhage. Black,[1] in his critical review of the subject, came to the conclusion that "the available evidence suggests that the usual moderate rise in blood urea is accounted for mainly by absorption of nitrogen in large amounts from the blood in the bowel when the kidneys are hampered by a diminished volume—flow of blood." Schiff and his co-workers,[5,6] on the other hand, were convinced from their observations on 53 cases, that the azotemia was previously due to absorption of blood proteins from the intestinal tract. Yuile and Hawkins[7] fed mongrel dogs blood fractions, such as, hemoglobin, and plasma proteins, and arrived at the same conclusions that Schiff did. Unfortunately, Yuile and Hawkins failed to bleed their animals before feeding blood components. This experimental work does not parallel the actual conditions occurring in individuals who bleed into their gastro-intestinal tract. Kaump and Parsons[3] simulated human gastro-intestinal bleeding in experimental animals. They bled dogs from the heart and fed the animals their own blood. They concluded that much remained to be clarified, although they were inclined to agree with Schiff that the azotemia was due largely to absorption of the protein (blood) from the intestines. Johnson[2] stated that the azotemia of gastric hemorrhage did not occur in patients in the absence of reduction in renal function.

It may be mentioned that Yuile and Hawkins found that the time required to reach the maximum blood urea N in mongrels was about 4½ to 10 hours after blood feedings. Kaump and Parsons observed the maximum rise to be about 24 hours. This approximated more closely the findings of Black and Schiff, *et al.* in human gastro-intestinal bleeding.

It was our purpose to attempt to correlate the different conclusions of these workers, and, if possible, to present experimental work nearly approaching the physiologic conditions existing in man by using human subjects. How much blood must be lost in the intestines to produce a significant azotemia in man? Does the question of a renal factor based on diminished volume flow enter into the picture, as propounded by Black—or is the phenomenon primarily one of alimentary azotemia? These were the questions to be answered.

Experimental. A. The authors and 3 of their medical corps associates acted as subjects. All were in normal health and under 40 years of age. Three to 5 days prior to the experiment, urea N, plasma proteins, phenols, hematocrit index, creatinine and uric acid determinations were performed on specimens of their blood. Routine urinalysis including microscopic examination, and a urea clearance test were carried out at the same time. The values obtained were all normal. Without any breakfast, and after a 10 hour fast, the men were bled to the amount of 580 cc. in 25 cc. of 5% sodium citrate solution. A venepuncture just prior to the large bleeding provided the necessary blood for repeating the above chemical studies. A few minutes after withdrawing the 580 cc. of blood, the subjects drank their own blood followed by 100 cc. of water. The ingestion was spread over a period of 30 minutes. Urea clearance, blood urea N, creatinine, blood phenols, and uric acid determinations were done every 3 hours for 12 hours following the ingestion of blood. The subjects were permitted an occasional peppermint candy, water, and smoke throughout this

12 hour period. They maintained their usual daily activities. A number of urinalyses during the experiment showed no abnormal elements in any of the specimens.

The psychic trauma was non-existent. Two officers complained of a taste of liver about 3 hours after feeding. No nausea, vomiting or diarrhea was reported. At the completion of the 12 hour period, a light supper was eaten, and no other food was ingested that night. The following morning, without breakfast (24 hours after blood ingestion), a complete repeat chemical examination of the blood was performed on each subject, plus a urea clearance test. Three hours later the same studies were again repeated. The usual lunch and supper were then permitted. Repeat blood and urine studies were performed 48 and 51 hours after the blood ingestion. Since little variation was encountered in the values obtained between subjects, they were averaged and the results appear in Figures 1, 2 and 3. Interestingly, during the experimental period only one subject had one loose tarry stool—all the others had black, formed stools throughout the investigation.

It is apparent from Figure 1 that the expected high elevation in blood urea N did not occur. The average maximum increase was small and could be accounted for by the ordinary rise due to large protein ingestion. The hematocrit index fell slightly, as was expected. The moderate increase in uric acid (Fig. 2) could be explained on the basis of the existence of nucleoproteins and uric acid itself in the whole blood. The fall in plasma proteins needs no discussion. The increase in phenols was probably due to the presence of phenolic-amino acids in the blood proteins. The urea clearance values in Figure 3 indicate a complete normal range for the group. However, one member showed a 68% of average normal function at the $6\frac{1}{2}$-hour mark. His previous clearances as well as those following were within normal limits.

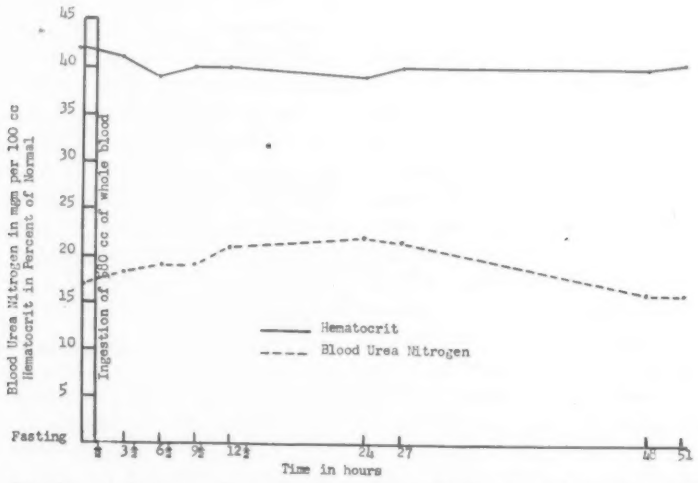

FIG. 1.—Blood urea nitrogen and hematocrit readings following the autoingestion of 580 cc. of whole blood. (Average values of 5 subjects.)

If one considers the amount of protein ingested in 580 cc. of whole blood, it is easily over 100 gm. of protein. This is more than the average adult eats in 24 hours.

There was the possibility that insufficient blood may have been removed to produce systemic effects similar to those observed in patients with massive hemorrhage into the gastro-intestinal tract. Three more medical officers, therefore, volunteered to donate 800 cc. of blood and undergo the studies carried out in A.

B. Blood ingestion, time intervals, chemical and microscopic examinations of the urine as well as chemical studies of the blood were carried out in exactly the same manner as in A except for the larger blood donation and ingestion. The subjects complained of vertigo and were pale. This restricted their normal activities to a minimum. The vertigo persisted throughout the day. It was no longer present the following morning. All subjects showed tarry stools within $3\frac{1}{2}$ hours after the blood ingestion. One individual passed 4 stools in a 14 hour period after the blood ingestion. The other 2 passed 3 tarry stools in the same interval. Within 32 hours all 3 subjects were passing black formed feces. The officer who had 4 bowel movements in 14

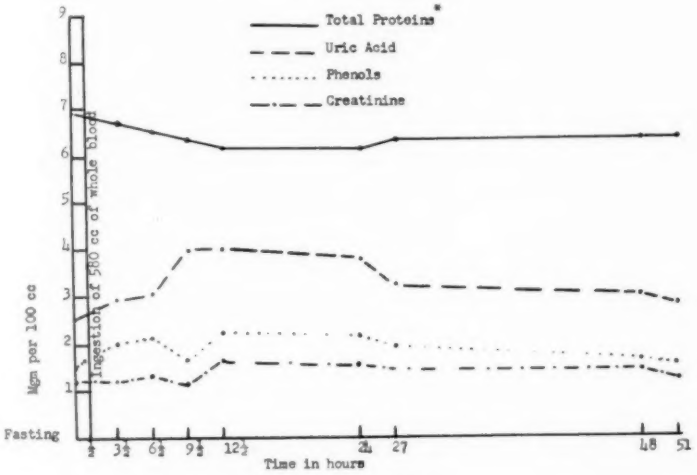

FIG. 2.—Blood total proteins,* uric acid, phenols and creatinine following the autoingestion of 580 cc. of whole blood. (Average values of 5 subjects.)

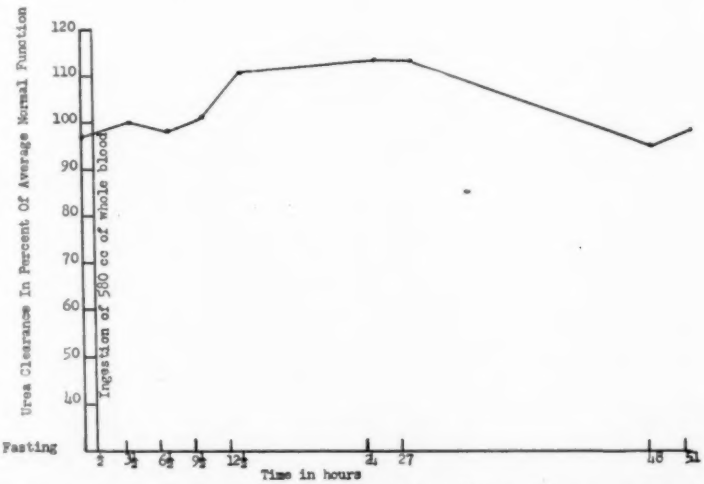

FIG. 3.—Urea clearance after autoingestion of 580 cc. of whole blood. (Average values of 5 subjects.)

hours continued to have a tarry stool at the 24 hour period. This could be termed a diarrhea, because none of the men habitually had more than 2 bowel movements a day.

protein, and very slight rise in creatinine. Because the individual variations were not significant, the results were averaged.

Although the quantitative difference in blood loss was only 220 cc. between Groups

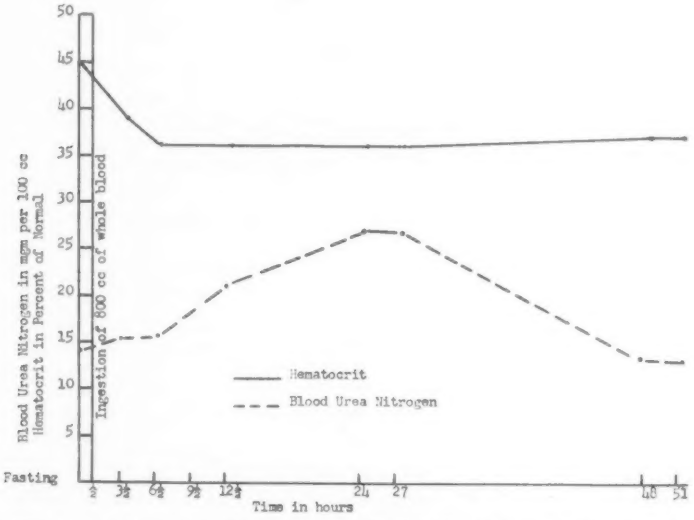
Blood Urea Nitrogen in mgm per 100 cc
Hematocrit in Percent of Normal
Ingestion of 800 cc of whole blood
Hematocrit
Blood Urea Nitrogen
Fasting
Time in hours

FIG. 4.—Blood urea nitrogen and hematocrit readings following the autoingestion of 800 cc. of whole blood. (Average values of 3 subjects.)

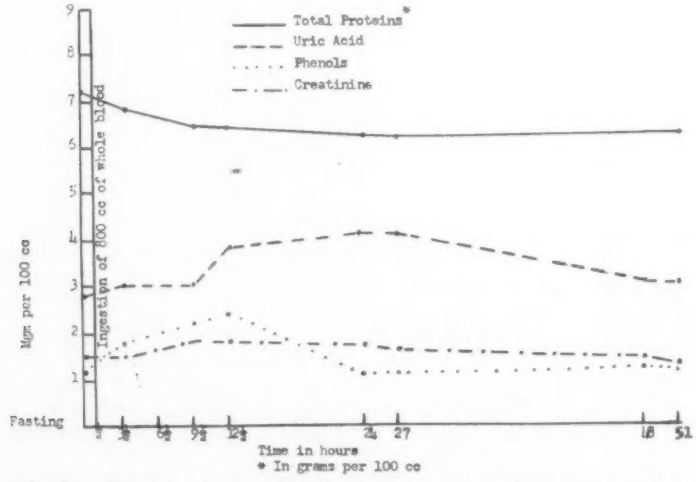

FIG. 5.—Blood total proteins,* uric acid, phenols and creatinine following the autoingestion of 800 cc. of whole blood. (Average values of 3 subjects.)

The findings in Figures 4, 5 and 6 show a definite azotemia, reduced urea clearance, fall in hematocrit index, rise in blood phenols and uric acid, fall in total

A and B, it appears that enough blood has been lost at the 800 cc. level to produce a picture similar to the azotemia in gastro-intestinal bleeding. The marked difference

in urea nitrogen figures and urea clearance findings in both groups is an indication of the disturbance established in renal function of the blood loss. The other values obtained for blood proteins, hematocrit index, uric acid, and blood phenols are not unexpected and do not vary much from those observed in Group A.

The maximum rise in blood urea N figures was observed to occur in both groups at 24 to 27 hours after the blood ingestion. This agrees with the results of Black and of Schiff, *et al.*

and particularly in urea clearance. To test this, another individual was fed a large beefsteak, the protein content of which was calculated at 159 gm., immediately after donating 500 cc. of blood for a transfusion. Certain technical difficulties arose making it impossible to obtain blood and urine specimens at the same intervals as in the previous experiments. We did manage to compare the fasting urea clearance and blood urea N before blood was withdrawn for the transfusion with those obtained 24 and 30 hours after the beef protein was

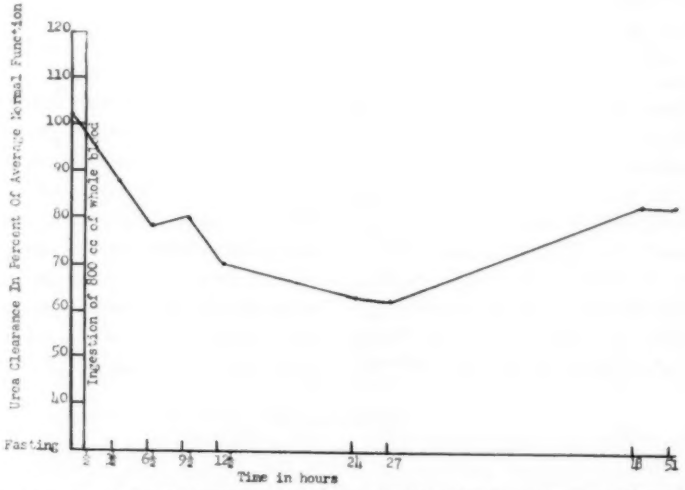

FIG. 6.—Urea clearance after autoingestion of 800 cc. of whole blood. (Average values of 3 subjects.)

Discussion. In the 3 subjects studied in Group B, a loss of 800 cc. of whole blood from the vascular system into the gastrointestinal tract produced definite metabolic changes. On the other hand, no such picture occurred in the 5 cases in Group A where 580 cc. of blood was lost into the stomach. It appears that a critical level exists between the 580 cc. and the 800 cc. blood loss. A point seems to be reached in circulating fluid dynamics where interference with renal function becomes manifest. We make this statement because we cannot conceive of 40 to 50 gm. increase in protein intake (based on the 220 cc. of increased blood ingested) causing such a marked change in blood urea nitrogen, eaten. The urea N rose to 22.3 mg./100 cc. from a fasting level of 16.1 mg./100 cc. The subject did not eat anything for 24 hours after the beefsteak was eaten. The blood urea clearance remained within normal limits in all the tests performed. Moon[4] has shown that in experimental hemorrhage the non-protein nitrogen is either unaltered or decreased. We, therefore, do not believe that bleeding alone can explain the marked urea nitrogen rise of Group B. The mild rise of urea N that occurred in Group A, and in the subject who ate beef protein after giving a transfusion can be explained as the ordinary increase expected following ingestion of a large amount of protein. On the other

hand, in Group B, the critical change in circulating fluid economy produced diminution in renal excretory function. This basic factor superimposed on the urea nitrogen increase due to large protein ingestion apparently causes the marked rise in urea nitrogen found in massive gastro-intestinal bleeding.

Summary. 1. A group of 5 normal adult males were each bled 580 cc. of blood and then drank the fluid. Periodic blood and urine examinations showed: (*a*) An average maximum increase in blood urea nitrogen of 24% above the fasting level, a slight decrease in plasma proteins and hematocrit index, a moderate increase in blood uric acid, phenols, and creatinine. (*b*) Normal urea clearance studies. (*c*) No systemic effects and black formed stools in nearly all cases.

2. A group of 3 adult normal males were each bled 800 cc. of blood and drank the fluid. Periodic blood and urine examinations revealed: (*a*) A marked elevation in blood urea N—90% above the fasting level, a moderate elevation of blood creatinine, phenols, and uric acid, a moderate fall in plasma proteins and hematocrit index. (*b*) A marked decrease in urea clearance compared to the experiments in A. (*c*) Moderate systemic effects for 24 hours.

Conclusion. Our results suggest a critical level at which blood lost from the vascular system and artificially "bled" into the intestinal tract produces no significant azotemia unless this level is exceeded. The level is somewhere between 580 and 800 cc. of whole blood. From this it appears that a gastro-intestinal hemorrhage greater than 580 cc. is necessary to produce the marked azotemia seen so frequently in bleeding peptic ulcers. Of course, the repeated loss at short intervals of a few hundred cc. of blood without compensatory regeneration, or else a slow, steady bleeding, would finally lead to a picture seen in acute massive hemorrhage into the bowel. The experimental work appears to support the contention of Black and of Johnson in that the azotemia of bleeding gastro-intestinal ulcers is primarily due to a fluid loss, sufficiently severe to impair normal renal function upon which is added the absorption of nitrogenous compounds from the enteral blood.

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A STUDY OF THE EFFECT OF BLEEDING AND OF REPEATED BLOOD DONATION ON SEROLOGIC TESTS FOR SYPHILIS*

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In December 1943 the Venereal Disease Subcommittee of the National Research Council recognized the impressions of several directors of Red Cross Donor Centers and other interested observers, to the effect that successive bleedings as such are capable of producing in some donors a non-specific positive reaction to recognized serologic tests for syphilis. To date we have seen no statistically valid data supporting this impression. Nevertheless the Subcommittee subsidized a special investigation. This was set up at the Institute for the Control of Syphilis, University of Pennsylvania, with the coöperation of the Philadelphia Red Cross Blood Donor Center. In its preliminary conferences, the group† charged with the work, broke down the project into two parts. In the first experiment, an unselected series of 98 seropositive donors was examined by all available means to determine the presence or absence of syphilis. This part of the study was essentially an examination of the positive serologic test with a view to its partitioning into specific and non-specific groups. From the non-specific group and from an examination of seropositive donors who had given more than one donation, it was thought that a clearly defined group of multiple donor biologic false or non-specific positive reactors might be identified, whose reactivity might reasonably be ascribed to successive bleedings. The study failed to support the presupposition. It was impossible to prove and equally impossible to deny the existence of a multiple donation non-specific positive phenomenon.

The results of this study have been accepted as a report and prepared for publication under the title, "Non-specific Reactions in Routine Blood Testing for Syphilis; a Study of the So-called Biologic False Postive Reactions."

The proportion of positive serologic reactors in the whole material, and of presumptive *non-specific* positive reactors was so small that the question might well be raised as to whether the positives observed in multiple donors might not be simply laboratory technical false positives, and not the reflex of an actual biologic false positive phenomenon in a multiple donor.

Moreover the elimination of syphilis and the establishment of a convincing cause in an individual case for a bona fide non-specific positive proved to be undertakings

* The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Pennsylvania.

† In addition to the authors of this presentation, the following group participated in these studies: A. Parker Hitchens, M.D., Herman Beerman, M.D., Norman R. Ingraham, Jr., M.D., Louise E. Tays, M.D., Virgene S. Wammock, M.D., Henry G. Morgan, Surgeon (R) USPHS, Stephen T. Whelan, M.D., Margaret J. Gerlach, M.D., on behalf of the Institute for the Control of Syphilis; Verner Nisbet, M.D., and Claude P. Brown, M.D., Director and Assistant Director respectively, Pennsylvania State Laboratory, Philadelphia; and the United States Public Health Service Venereal Disease Research Laboratory, John F. Mahoney, Medical Director, United States Public Health Service, Staten Island, New York.

of such extreme difficulty, that it was decided that no valid answer to the question could be had from the clinical-laboratory study short of years in time and millions in clinical case material.

It was therefore decided to set up Experiment 2 as a more controllable smaller-scale attack on the problem. A stable population source (prison inmates)* most of whom had given blood repeatedly, was bled under controlled conditions, and the serologic results collected. This material is the subject of the following report.

In order to conduct an investigation of the problem of the influence of bleeding on the serologic testing of blood, the American Red Cross Blood Donor Center arranged for a mobile unit trip to a nearby prison, thereby insuring a static group of donors which could easily be contacted again for follow-up. All donors at the institution had been previously screened by the prison physician in order to eliminate clinically those with recognizable syphilis.

One hundred sixty-four donors were accepted for bleeding, all of them men. Of these 27 were Negroes, the rest Whites. The ages varied from 18 through 58, preponderance of donors being between the ages of 21 and 40 years (Table 1).

TABLE 1.—AGES OF DONORS

Age	No.
18–20	1
21–25	40
26–30	40
31–35	25
36–40	31
41–45	15
46–50	7
51–55	2
56–60	3

Most of the donors had given blood before (see Table 2). For only 21 donors was this the first experience. Forty-three donors had donated blood more than 10 times.

The bleeding procedure used throughout was the prescribed procedure used in all Blood Donor Centers of the American Red Cross. The area chosen for venepuncture was scrubbed with green soap followed by alcohol. A 2% aqueous solution of iodine was then applied and when it had been allowed to dry, was removed with alcohol. Procaine, 1%, was injected intracutaneously at the chosen site of venepuncture and after a few minutes actual venepuncture was made with a 15-gauge needle.

TABLE 2.—NUMBER OF DONATIONS GIVEN BY INDIVIDUALS

Times	No.
1	21
2	21
3	10
4	8
5	15
6	10
7	13
8	8
9	10
10	5
More than 10	43

As soon as bleeding was started, the lower end of the inlet tube was scrubbed with iodine and alcohol and a sterile 20-gauge needle inserted through this scrubbed area into the lumen of the tube. In this way, 10 cc. of whole blood was withdrawn into a syringe at the beginning of each venesection. The bleeding then continued in its normal fashion until the donor had given 500 cc. of blood at which time the bleeding bottle was clamped off, the tourniquet released and the needle removed from the vein. That amount of blood left in the inlet tube was then milked into a test tube to give us 8 to 10 cc. of whole blood. By following the above procedure we were able to collect samples of blood for study at the beginning and at the end of each venesection.

It is interesting to note here, that the procedure of inserting a sterile needle through cleansed rubber tubing in no way affected the sterility of the pint of blood withdrawn into the bleeding bottle. On checking with the processing laboratory, it was found that no contaminations had occurred in this shipment.

* We are indebted to Dr. Verne Burden, Medical Director and Chief Surgeon of the Eastern State Penitentiary, Philadelphia, and to the coöperating inmates, in this study.

All samples of blood taken at the end of venesection were sent to the processing laboratory where routine serologic testing was employed. This consisted of a Kahn and only those showing reaction were then followed up with a Kolmer. Serum that remained in sufficient quantity was then returned to us for study. We therefore had 128 samples that could be compared with those taken when venesection was begun. All reports from the processing laboratory were negative except one which was reported as Kahn plus 2, Kolmer plus 3.

On the 5th day following the donation of 500 cc. of blood, 10 cc. samples of blood were withdrawn from 38 of these donors (Group 1) chosen at random. Ten cc. samples were again withdrawn from the donors in this group on the 9th and 13th days.

Another group of 35 donors (Group 2) was seen on the 7th day after their donations of a pint of blood. From these too, we withdrew 10 cc. of whole blood and repeated this procedure on the 11th and 15th days.

Group 3 consisted of 24 donors tested once or twice from the 5th to the 15th day. The results obtained in Groups 1, 2 and 3 are shown in Table 1.

Serologic Procedures. Two tests were used in this experiment, one a *quantitative flocculation test* and the other a *quantitative complement fixation test.* The B.J.L. Macro flocculation test modified to detect at least $\frac{1}{4}$ of a unit was used. This technique is a modification of Lund's technique for determining small amounts of antibody (reagin), and is the same as that described by Boerner, Jones and Lukens except three dilutions of antigen were employed. Three tubes were used: the first carried 0.25 cc. of serum and 0.05 cc. of antigen emulsion; the second tube was the same except the antigen was diluted with an equal volume of saline before using; the third carried antigen that had been diluted to 4 times its volume with saline. Reactions in the first tube were given the value of 1 unit; in the second tube $\frac{1}{2}$ unit, and in the third tube $\frac{1}{4}$ unit. Failure to obtain a reaction in any tube was recorded at no units which in this experiment means less than $\frac{1}{4}$ unit. We did not believe the detection of less than $\frac{1}{4}$ unit would be of any value in this study.

The quantitative complement-fixation technique employed was designed to determine the ability of serum to fix complement and differs from the usual methods in that the amount of complement was varied and the serum dose kept constant. We have called this technique the *fixation of complement test* to distinguish it from other quantitative complement fixation tests which titer the serum. The technique is similar to that used by Boerner and Lukens except three dilutions of complement (1:40, 1:60, 1:80) were substituted for the 1:30 dilution in the test itself. When fixation occurred in the 1:40 dilution, the test was repeated using eight dilutions of complement (1:10, 1:15, 1:20, 1:30, 1:40, 1:60, 1:80, and 1:120). For each dilution of complement a serum, antigen and hemolytic control was included.

The units of fixation are determined by reading and recording the amount of inhibition of hemolysis in each tube according to Table 3:

TABLE 3.—AMOUNT OF INHIBITION OF HEMOLYSIS

Amount, %				Unit of fixation
100	=	+4	=	1
75	=	+3	=	$\frac{3}{4}$
50	=	+2	=	$\frac{1}{2}$
25	=	+1	=	$\frac{1}{4}$
$12\frac{1}{2}$	=	*	=	$\frac{1}{8}$
Under $12\frac{1}{2}$	=	−0	=	0

Beginning with the strongest dilution of complement which shows at least $12\frac{1}{2}$ % inhibition of hemolysis in the serum control tube and no inhibition in the antigen control, calculate the specific fixation by subtracting the amount of inhibition in the serum control tube from the tube carrying antigen, using the values given above. To this figure add all the units of fixation in the tubes with stronger dilutions of complement.

Figuring from the 1:80 dilution, the

units in the serum and the antigen tubes would be $4\frac{1}{2}$ and the units in the controls $\frac{1}{8}$. The final reading would be $4\frac{1}{2} - \frac{1}{8}$ or $4\frac{3}{8}$ units.

Experimental Material. A group of 164 donors was tested immediately before the withdrawal of blood, and 128 of these were tested at the end of bleeding to determine if the removal of 500 cc. of blood would cause any immediate change in the serologic tests.

Ninety-seven of the above group were tested at various intervals after donation to determine if any changes in the serologic tests occurred. In this study the donors are grouped into 3 groups as follows:

Group 1. Consisted of 38 donors tested on the 5th, 9th and 13th day following donation.

Group 2. Consisted of 35 donors tested on the 7th, 11th and 15th day following donation.

Group 3. Consisted of 24 donors tested once or twice from the 5th to the 15th day. The results obtained in Groups 1, 2 and 3 are shown in Table 1.

Results of the Fixation of Complement Test. In presenting the results of this study we have allowed $\frac{1}{2}$ a unit in the fixation of complement test for experimental error. Therefore, results reported as no units include all those having $\frac{1}{2}$ or less units, and the units given in other cases are the number in excess of $\frac{1}{2}$ unit.

The *original test* before bleeding (April 5, 1944) showed:

151 with $\frac{1}{2}$ or less units
6 with $\frac{1}{4}$ to 2 units
3 with $2\frac{1}{2}$ to $3\frac{1}{8}$ units
4 with 4 to $5\frac{1}{2}$ units

The 4 with high units failed to donate for the follow-up tests. It was later ascertained that at least 2 were old cases of syphilis. On May 15 we succeeded in obtaining blood from these 4 for re-testing. The following gives the results of the original test and the re-test on May 15:

No.	Original April 6 (units)	Re-test May 15 (units)
41103	5	$5\frac{1}{2}$
41104	5	$4\frac{3}{4}$
41107	4	$3\frac{3}{4}$
41075	$5\frac{1}{4}$	$2\frac{1}{4}$

The repeated test performed on 128 specimens collected immediately after bleeding showed 119 to give reactions identical with the original test before bleeding; 8 were within $\frac{1}{2}$ unit and 1 showed a difference of 1 unit.

The results obtained in Group 1 are shown in Table 5. Of the 38 donors tested, only 4 showed $\frac{1}{2}$ or more units in one or more of the tests. One (No. 41037) showed an increase in units having $\frac{1}{2}$ unit in the original test and $2\frac{1}{2}$ units 11 days later. This donor was subsequently tested on May 15, 1944, and showed $1\frac{1}{2}$ units.

The results obtained in Group 2 showed no increase in units from the 7th to 15th days after donation (Table 5). The 3 donors (Nos. 41073, 41113, and 41173) which showed 2 to 3 units were tested on May 15 (see Table 6).

The results in Group 3 are given in Table 5. All donors in this group gave $\frac{1}{2}$ or less units before and after donation of blood.

Results of the Flocculation Tests. In the original test before donation (April 6) of 164 donors all showed less than $\frac{1}{4}$ unit of flocculation with the exception of two (Nos. 41073 and 41077). A repeat test on 128 of these donors immediately after donation gave results identical with the original test before bleeding.

The re-tests of Groups 1, 2 and 3 were the same as the original and are shown in Table 3.

A number of these donors gave 2 to $5\frac{1}{4}$ fixation of complement units with less than $\frac{1}{2}$ unit of flocculation. Five of these failed to donate for the follow-up tests. On May 15 these donors were re-tested and the results are shown in Table 4. A record of at least 5 of these donors showed them to be old cases of syphilis.

The assumption that all these donors were non-syphilitic was based principally on the fact that they had given a number of donations with negative serologic reports. The results in Table 4 shows the inadequacy of using a single test as a screen. However, none of these cases was in the early stage of the disease and it is

unlikely that many early cases would be missed by screening.

On the other hand, it gives support to those who believe that at least 2 tests should be used as a diagnostic procedure and 1 of them a complement-fixation test.

Conclusions. 1. Blood donors (128) were tested by a quantitative flocculation and complement-fixation test for syphilis, immediately before and immediately after bleeding. No significant difference in reagin content of the serum (antibody) was observed.

2. Blood donors (97) were tested for flocculation and complement-fixing reagin (antibodies) for syphilis before and at in-

TABLE 4.—EXAMPLES OF COMPLETED TEST

Complement	Serum antigen	Serum control	Antigen control	Hemolytic control
1:10	−	−	−	−
1:15	−	−	−	−
1:20	+2	−	−	−
1:30	+4	−	−	−
1:40	+4	−	−	−
1:60	+4	−	−	−
1:80	+4	±	−	−
1:120	+4	+3	+1	−

TABLE 5.—RESULTS OBTAINED IN 3 GROUPS

		Before donation		After donation: 5 days		7 days		9 days		11 days		13 days		15 days	
Group	Donor	Floc (un.)	F-C (un.)	Floc (un.)	F-C (un.)	Floc (un.)	F-C (un.)	Floc (un.)	F-C (un.)	Floc (un.)	F-C (un.)	Floc (un.)	F-C (un.)	Floc (un.)	F-C (un.)
1	41115	0	1	0	0	..	..	0	1	..	..	0	1½		
	41138	0	½	0	0	..	..	0	1	..	..	0	½		
	41037	0	½	0	0	..	..	0	1½	..	..	0	2½		
	41163	0	0	0	0	..	..	0	0	..	..	0	¼		
	34 donors	0	0	0	0	..	..	0	0	..	..	0	0		
2	41073	¼	2	..	..	½	2	..	..	½	¾	..	..	¼	2
	41095	0	0	..	..	0	¼	..	..	0	0	..	..	0	0
	41112	0	0	..	..	0	¼	..	..	0	0	..	..	0	0
	41113	0	3	..	..	0	2½	..	..	0	2½	..	..	0	3
	41173	0	2¼	..	..	0	2	..	..	0	2¼	..	..	0	2
	41177	0	0	..	..	0	¼	..	..	0	0	..	..	0	0
	29 donors	0	0	..	..	0	0	..	..	0	0	..	..	0	0
3	41109	0	0	..	..	0	¼								
	41049	0	0	..	..	0	½								
	9 donors	0	0	..	..	0	0								
	4 donors	0	0	..	..	0	0	..	..	0	0				
	4 donors	0	0	0	0	..	..	0	0						
	2 donors	0	0	0	0										
	1 donor	0	0	..	..	..	..	..	..	..	..	0	0		
	1 donor	0	0	..	..	..	..	..	..	0	0				

TABLE 6.—ORIGINAL AND REPEATED TESTS ON SEROLOGIC POSITIVE DONORS

		April 6, 1944		May 15, 1944				
Case No.	No. donations*	Receiving station†	Floc (un.)‡	C-F (un.)‡	Floc (un.)‡	C-F (un.)‡	Kahn§	Kolmer§	Remarks
41103	15	Kahn-neg.	0	5	0	5¼	Neg.	Pos. +4	
41104	11	Kahn +2 Kolmer +3	0	5	0	4¼	Neg. ±	Pos. +4	Old treated syphilis
41107	2	Kahn-neg.	0	4	0	3¾	Neg.	Pos. +3	
41075	7	Kahn-neg.	0	5¼	0	2¾	Neg.	Pos. +4	Old treated syphilis
41073	16	Kahn-neg.	0	2¼	0	3¼	Neg.	Neg.	Treated syphilis
41077	5	Kahn-neg.	0	2¾	0	3¾	Dbt. +	Pos. +4	Old treated syphilis
41113	5	Kahn-neg.	0	3	0	1½	Neg.	Neg.	
41073	13	Kahn-neg.	¼	2	0	¼	Neg. ±	Anticomp.	Old treated syphilis

* Previous donations to the Red Cross Blood Bank with negative Kahn tests.
† Tests performed at the processing laboratories.
‡ Tests performed at the Graduate Hospital, University of Pennsylvania.
§ Test performed at the United States Public Health Laboratory, Staten Island, New York.

tervals from the 5th to the 15th day following donation. One showed an increase of 2 fixation of complement units and none showed any increase in flocculation units.

3. Within the limits imposed by the conditions of the experiment, including the limited number of donors used, and by possible confusing elements such as intercurrent infections or other known causes of biologic false positives, and the fallibilities of the clinical examination for syphilis as performed, it can certainly not be said that this series reveals the existence of a non-specific positive due to multiple blood donation, or to bleeding as such. Neither can it be said that such a possibility is totally excluded.

4. No statistically valid data are yet known to the investigators of this group which support the hypothesis that successive bleedings are capable of producing in some donors a non-specific positive reaction to recognized serologic tests for syphilis.

HEREDITARY HEMORRHAGIC TELANGIECTASIA

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Hereditary hemorrhagic telangiectasia is a definite clinical entity characterized by typical localized dilatation of small vessels to form angiomatous lesions, and a tendency to bleed from these areas. Telangiectases occur most commonly on the skin of the face and neck, and on the buccal and nasopharyngeal mucous membranes. It is an hereditary disease, being transmitted as a simple dominant. Both sexes are affected and both transmit the disease.

Babington[1] in 1865 described hereditary epistaxis. Rendu[14] in 1896 reported a case of telangiectasia, and recognized the pathology and the hereditary aspect of the disease. At the turn of the century Sir William Osler[11,12] did a great deal to establish telangiectasia as a clinical entity and to make it familiar to the profession. The literature on hereditary hemorrhagic telangiectasia was summarized by Goldstein[7] in 1931, by Houser[8] in 1934, and by Stock[16] and by Barrock[2] in 1944.

The diagnostic criteria of hereditary hemorrhagic telangiectasia were established by Goldstein[7] and affirmed by Larrabe and Littman.[9] These are: definite hereditary tendency, visible typical telangiectases and a tendency to bleed from these lesions. A normal bleeding time, clotting time, clot retraction, platelet count and tourniquet test are usually considered essential for the diagnosis.

The lesion in this disease has been thought of as a local developmental defect of small vessels. Singer and Wolfson,[15] in their study of a family with telangiectasia, suggested that a more fundamental capillary disturbance may be present. This hypothesis was based on the fact that their cases, in addition to typical telangiectasia, showed the unusual feature of a positive tourniquet test. Singer and Wolfson[15] classify them as pseudohemophilia, hereditary familial purpura simplex and hereditary hemorrhagic telangiectasia. Pseudohemophilia (von Willebrand[17]) is characterized by prolonged bleeding time, normal clotting time and clot retraction, a normal platelet count and a variable tourniquet test. Hereditary familial purpura simplex[4,5] presents a positive tourniquet test, but a normal bleeding time. The characteristics of telangiectasia have been outlined.

In addition to the pure types of hereditary capillary bleeding syndromes described above, cases of one are seen with some of the features of another. The reported combinations of these diseases are depicted in Figure 1. This diagram is taken from Singer and Wolfson.[15]

Cases of pseudohemophilia are seen with a positive tourniquet test (indicating decreased capillary resistance) and also at times with deficient clot retraction; therefore, overlapping with hereditary familial purpura simplex. Singer and Wolfson's[15] cases of hereditary hemorrhagic telangiectasia showed evidence of decreased capillary resistance (positive tourniquet test); hence, the overlap between hereditary familial purpura simplex and telangiectasia (Fig. 1).

As can be seen in Figure 1, there is no overlap between telangiectasia and pseudohemophilia, no case of telangiectasia with prolonged bleeding time having been reported previously. In their discussion of the capillary heredopathies, Singer and Wolfson[15] predicted the possibility of the occurrence of a prolonged bleeding time in hereditary hemorrhagic telangiectasia.

The purpose of this paper is to report 5 cases of hereditary hemorrhagic telangi-

ectasia all fulfilling the diagnostic criteria of Goldstein.[7] In addition, they present various unusual associations. Two of these cases show prolongation of the bleeding time, thus fulfilling the prediction of Singer and Wolfson.[15]

The patient's parents were first cousins. Two maternal aunts had frequent epistaxes and menorrhagia. They were said to have died of hemorrhage from the nose. A maternal uncle had frequent epistaxes most of his life and died of hemorrhage from the

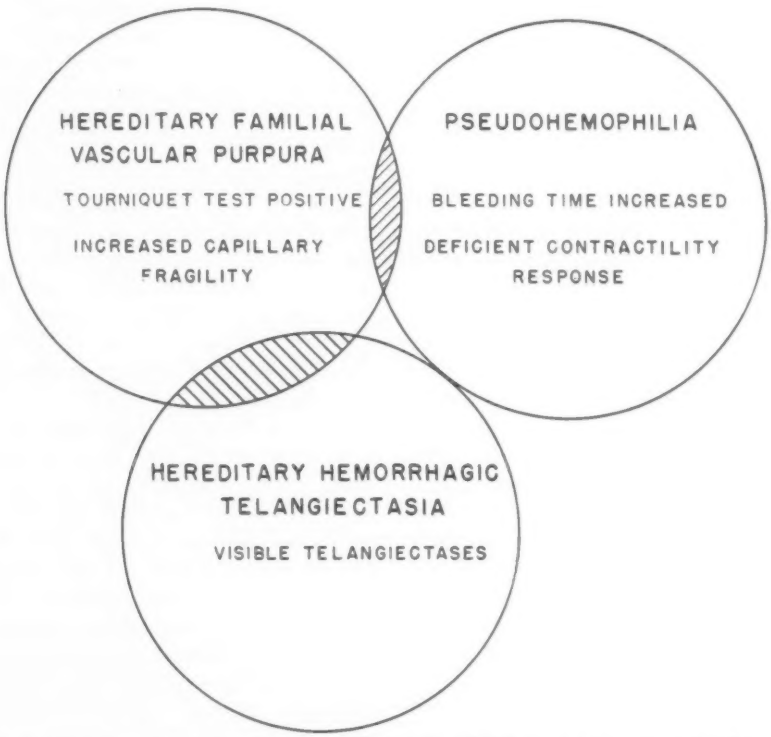

FIG. 1.—Diagram illustrating the various pure types and the reported combinations of hereditary capillary syndromes. The shaded areas represent the observed combinations. (Reproduced through the courtesy of the New England Journal of Medicine.)

Case Reports. CASE 1. A white, single female, aged 57, had frequent epistaxes and bleeding from the mouth as long as she could remember. Since the onset of her menses, there had been severe menorrhagia. Nine years before admission she had a profuse vaginal hemorrhage causing weakness and pallor. Roentgen ray sterilization was done with cessation of menorrhagia.

Three to 4 weeks before admission she began bleeding from the nose and mouth and vomited large amounts of blood. The day before admission uncontrollable hemorrhage from the nose occurred. She became weak and pale, and was admitted to the hospital in a state of shock.

nose. One brother, aged 65, had numerous nosebleeds throughout most of his life until 10 years ago.

Physical examination on admission revealed the signs of shock due to blood loss, for which she was successfully treated. The skin was pale, and showed several purpuric areas over both lower extremities. On superficial examination no telangiectases were seen. The liver and spleen were not palpable. Otolaryngologic consultation revealed fairly large dilated thin-walled veins in the region of the posterior nares. These were more marked on the left side, and the left turbinate was very hyperemic.

Laboratory studies on admission showed a red blood cell count of 1,340,000 per c.mm., a white blood cell count of 18,850 per c.mm., and a hemoglobin of 5 gm. %. Following a series of transfusions the red blood cell count was 4,890,000 per mm. and the hemoglobin 12.4 gm. %. The blood smear was normal with an abundance of platelets. The bleeding time on 2 occasions was 6 minutes and 6½ minutes, respectively. The coagulation time on 4 determinations was normal, ranging from 1 to 5 minutes. Clot retraction on 2 occasions was satisfactory in from 2 to 3 hours. One week later clot retraction was poor after 24 hours. The tourniquet test on 2 occasions showed 16 and 18 petechiæ respectively per 2.5 cm. area. The prothrombin time was normal (method of Quick) and the plasma fibrinogen was 0.36 gm. %.

This case presented all the criteria for the diagnosis of hereditary hemorrhagic telangiectasia, *i. e.*, an hereditary tendency, visible telangiectases, and bleeding from these lesions. In addition, a prolonged bleeding time, and a positive tourniquet test were present. Clot retraction was variable.

Case 2. A white female, aged 40, had no hemorrhagic tendency prior to 9 years before admission when she had severe bleeding from the nose lasting several days. This was associated with weakness and pallor. At intervals from that time until admission she had profuse epistaxes and several times had spontaneous hemorrhage from the mouth. Six years before admission she experienced a severe postpartum hemorrhage, but had no excessive bleeding following several other deliveries. For 1½ years prior to admission she had irregular menstrual periods associated with menorrhagia. Very frequent vaginal bleeding was present for 3 months prior to admission. She entered the hospital bordering on shock from blood loss due to hemorrhage from the uterus.

The patient's father and 1 paternal aunt had frequent profuse epistaxes throughout most of their lives. Three of 4 of the patient's children have excessive hemoptyses.

Physical examination on admission revealed her to be very pale with cold, moist skin and a rapid, thready pulse. The blood pressure was normal. Scattered over the skin of the face, neck, arms and thorax were numerous telangiectases. Definite dilated vessels were seen in the mucosa of the nasal septum, and several small telangiectases were present on the hard palate. The liver was felt 3 cm. below the costal margin in the right midclavicular line. The spleen was easily felt 3 cm. below the costal margin.

Laboratory studies on admission revealed the red blood cell count to be 1,300,000 per c.mm. and the hemoglobin was 2.7 gm. %. The white blood cell count was 7500 per c.mm. The blood smear showed a scarcity of platelets. A platelet count was 46,800 per c.mm. The anemia was corrected with transfusions of whole blood. Four blood smears while the patient was in the hospital showed a paucity of platelets. Seven platelet counts ranged from 3000 to 46,800. Other blood studies are shown in Table 1.

The tourniquet test was completely negative on 1 occasion and 15 months later showed 12 petechiæ per 2.5 cm. area, the same degree of hemostasis being used both times. Clot retraction was satisfactory on 1 occasion, and poor on 2 others. The clotting time was normal while under observation in the hospital, and definitely prolonged 15 months later.

After discharge from the hospital she had no more epistaxes and only slight menorrhagia. Fifteen months after discharge her red blood cell count and hemoglobin were normal. Other blood studies are shown in Table 1.

This case demonstrated hereditary hemorrhagic telangiectasia with a consistently prolonged bleeding time. In addition there was definite thrombocytopenia.

Case 3. A white female, aged 49, gave a history of frequent epistaxes and hemoptyses for many years. The patient's grandmother, mother, 1 maternal aunt, 2 siblings, and her 2 children all had frequent epistaxes. Several of the above relatives were examined and found to have typical telangiectases over the skin of the face, neck and on the nasal mucous membrane.

At age 18 the patient had migrating polyarthritis associated with fever and weakness.

On *physical examination*, scattered linear, spider-like and nodular telangiectases were seen over the thorax, arms, neck and face. In the skin over the right arm were several small purpuric spots. Dilated vessels were

seen in the nasal mucosa. A few medium moist râles were heard at the left lung base. The heart was markedly enlarged, with physical signs of mitral stenosis and insufficiency. The liver and spleen were not felt.

Laboratory findings included a red blood cell count of 4,400,000 per c.mm., a white blood cell count of 6200 per c.mm., and a hemoglobin of 13.2 gm. %. The blood smear appeared normal with adequate numbers of platelets. Platelet counts were normal. The bleeding time was 3 minutes and the clotting time was 5½ minutes. The tourniquet test showed innumerable petechiæ after 10 minutes with the cuff inflated to a pressure midway between the systolic and diastolic blood pressures. Repeated sputum examinations showed no acid-fast organisms. Roentgenographic examination of the chest including bronchograms revealed no pulmonary lesion.

A family history was obtained of frequent epistaxes in the patient's son, 2 brothers, 1 sister and several nephews.

On *physical examination*, numerous telangiectases were found on the face. Dilated vessels were seen on the right side of the nasal septum and a plexus of dilated vessels was present at the posterior tip of the left inferior turbinate. The liver and spleen were palpable.

Laboratory studies included a red blood cell count of 5,200,000 per c.mm. and a white blood cell count of 6550 per c.mm.; hemoglobin was 12 gm. %. The blood smear was normal with an abundance of platelets. The bleeding time was 2.5 minutes and the clotting time was 2 minutes. Clot retractility was normal. The tourniquet test was negative and the platelet count was 721,000 per c.mm.

Examination of the patient's son revealed

TABLE 1.—STUDIES ON CASE 2 IN HOSPITAL AND 15 MONTHS FOLLOWING DISCHARGE

Date	Bleeding time (min.)	Clotting time (min.)	Clot retraction	Tourniquet test
Jan. 4	4 5	4 0	Satisfactory	
Jan. 14	7 0	7-8	None at 12 hr.; slight at 24 hr. (room temp.)	No petechiæ after 30 min. at 50 mm. Hg pressure
Jan. 19	6 5	2 5		
April 2 1 year later	11 0	14 0	Poor	12 petechiæ in 20 min. at 50 mm. Hg pressure
April 30 1 year later	5 0	16 0		

The patient has been seen in the clinic at intervals for the past 7 years since discharge from the hospital. There has been a decrease in the hemorrhagic tendency, and she is being treated for mild chronic congestive heart failure.

This case presented a classical picture of hereditary hemorrhagic telangiectasia. In addition, increased capillary fragility, shown by a positive tourniquet test, was present. Associated was rheumatic heart disease.

CASE 4. A white female, aged 46, was admitted to the hospital with a history of repeated nosebleeds since the age of 10. Nine years before admission a hemorrhage from the nose occurred necessitating transfusion. Since the onset of menses she had had severe menorrhagia.

well-marked telangiectases on the face and left side of the nasal septum.

This is a typical case of hereditary hemorrhagic telangiectasia.

CASE 5. An electrician, aged 33, was admitted to the hospital because of bleeding from the throat. He gave a history of frequent epistaxes beginning at the age of 15. Lesions on the lower lip that would bleed at frequent intervals were noted by the patient as long as he could remember. Eight days before admission he began to bleed from the mouth and cough up large clots of blood. This persisted until admission.

He gave a family history of frequent hemorrhages from the mouth and nose in his daughter, his father, 1 brother and 1 sister.

On *physical examination* there were striking telangiectases on the lower lip and on

the posterior pharyngeal wall. Excoriated areas were seen on both sides of the nasal septum. The remainder of the physical examination was essentially negative.

Laboratory findings showed a red blood cell count of 4,850,000 per c.mm., a white blood cell count of 9000 per c.mm. and a hemoglobin of 11.2 gm. %. The blood smear was normal with adequate numbers of platelets. The bleeding time was 1 minute and the clotting time was $2\frac{1}{2}$ minutes. Clot retraction was slight at 24 hours. The tourniquet test revealed an average of 10 petechiæ in a 2.5 cm. circle after 15 minutes with the blood pressure cuff midway between the systolic and diastolic pressures.

This case presented classical hereditary hemorrhagic telangiectasia associated with poor clot retraction and a borderline tourniquet test.

Comment. Five cases are presented, all satisfying the diagnostic criteria of hereditary hemorrhagic telangiectasia. In addition, 2 of these showed prolongation of the bleeding time. The coagulation time in 1 case was prolonged on 2 occasions, and normal on several others. Clot retraction was variable in 2 patients. The tourniquet test was positive in 2 cases, equivocal in 1, variable in 1, and negative in 1. Platelets were described as numerous in 2 of these patients, normal in 2, and definitely decreased in 1 case.

A normal bleeding time, clotting time, clot retraction, platelet count, and the tourniquet test are usually found present in hereditary hemorrhagic telangiectasia. Classification of bleeding syndromes in tables of differential diagnosis[13,18] are based largely on the above tests. That no such rigid form of classification is applicable has been pointed out.[6,15]

Cases 1 and 2 present all the criteria for the diagnosis of hereditary hemorrhagic telangiectasia. In addition, the bleeding time was prolonged in both cases. Singer and Wolfson[15] presented an analysis of the

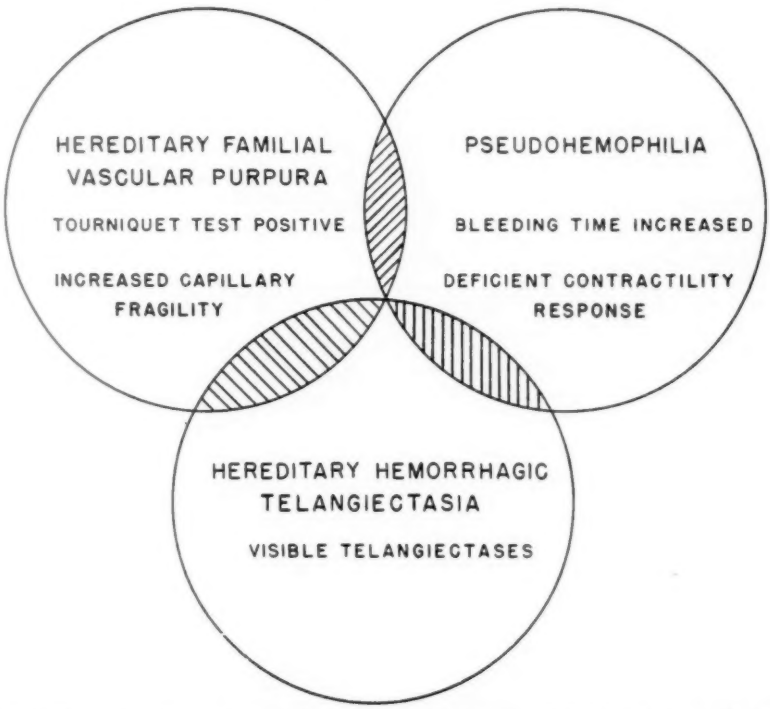

FIG. 2.—Modification of Figure 1 illustrating more complete interrelationship between the hereditary capillary syndromes.

pathologic physiology of the capillary bleeding syndromes. They regarded an abnormal response of a capillary to injury[10] (expressed by a prolonged bleeding time), and increased capillary fragility (expressed by a positive tourniquet test) as unrelated abnormalities that may occur independently or together. These two abnormalities exist independently in the pure types of pseudohemophilia and hereditary purpura simplex. The abnormalities exist together in the reported combinations (see Fig. 1). Singer and Wolfson[15] found cases of telangiectasia with a positive tourniquet test (increased capillary fragility) and reasoned by analogy that telangiectasia with a prolonged bleeding time (defective capillary retractility)[10] could occur. Cases 1 and 2 fulfill their prediction. The interrelationship shown in Figure 1 may now be completed as shown in Figure 2.

It is seen that definite thrombocytopenia is present in Case 2. In the presence of thrombocytopenia, to attribute prolonged bleeding time to deficient capillary contractility may invite criticism. However, the exact mechanism of the platelets in hemostasis is not known and there is not always a correlation between thrombocytopenia and increased bleeding time.[3,10] The tourniquet test in this case was negative in association with thrombocytopenia and a prolonged bleeding time. This is evidence of deficient capillary contractility.[10]

Cases 1 and 2 showed a definitely positive tourniquet test. In Cases 2 and 5 the tourniquet test was borderline. Singer and Wolfson[15] pointed out the association of increased capillary fragility in association with typical telangiectasia, reporting 3 cases. They could find only 1 family in the literature of hereditary hemorrhagic telangiectasia associated with a positive tourniquet test. This family had hereditary familial purpura simplex associated with telangiectasia and was reported by Davis.[4]

In Cases 1 and 2 there was variability in some of the tests from one time to another. The clot retractility in both cases was satisfactory on some occasions and deficient on others. In Case 2 the clotting time varied, being normal while the patient was in the hospital and definitely prolonged 15 months later. The tourniquet test in Case 2 was negative on 1 occasion and borderline on another (Table 1). These variations in the hereditary bleeding syndromes have been observed and emphasized.[6]

Summary. 1. Five cases of hereditary hemorrhagic telangiectasia are presented.

2. Deficient capillary contractility and decreased capillary resistance are observed in association with telangiectasia.

3. Variations in other tests of the hemostatic mechanism usually considered normal in telangiectasia are observed in this disease.

4. That hereditary hemorrhagic telangiectasia is a fundamental capillary disturbance is supported.

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BRONCHOPULMONARY GEOTRICHOSIS

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According to Dodge,[4] geotrichum is a sub-species of eremnascaceæ Imperfectæ, and was first described by Link in 1809. In many respects it resembles blastomyces dermatitidis and coccidiodes immitis. It has frequently been confused with the more pathogenic blastomyces dermatitidis.[9] The genus is quite large, the species being mostly saprophytic on earth and decaying organic matter. According to Dodge,[4] until 1935 12 cases had been reported in man, 8 from infections of the respiratory tract, 3 from stools, parasitism unknown, and 2 from blastomycosis.

In 1933 Smith[9] reported 5 non-fatal infections due to geotrichum and gave the essential cultural characteristics necessary for identification of the fungus. He emphasized that patients with clinical symptoms of chronic bronchitis or pulmonary tuberculosis, who have white mucoid sputum containing grayish flakes, should be suspected of having an infection with the yeast-like or mold-like fungus geotrichum. The organisms can be cultured from the sputum on Saboraud's media, and identified by the rectangular conidia.

deAlmeida and DaSilva[3] recently isolated 4 strains of geotrichum from the sputum of patients presenting pulmonary symptoms, and they discussed the frequency of false yeast isolated from sputum of patients with similar symptoms.

Of 79 cases of bronchopulmonary mycosis reported by Reeves[8] recently only 1 was a case of geotrichum infection. Recent excellent articles by Gill,[5] Johnston and Heydemann[6] and Peterson[7] on pathogenic molds and the lesions they produce in the respiratory tract, failed to mention infection by geotrichum.

We are reporting our case not only because bronchopulmonary geotrichosis is of rare occurrence, but because we believe that any light that can be thrown on the recognition of any of the fungus diseases will curtail erroneous diagnoses of pulmonary tuberculosis and non-specific pulmonary disease.

Case Report. This 22 year old colored soldier was admitted to Ashford Geueral Hospital on July 16, 1944 with the following history: he entered the Army on Dec. 2, 1942 and felt well until the early part of Feb., 1944 when he complained of sharp pains in his left lower chest which disappeared in a few days.

This soldier had been with a cavalry regiment stationed in Western Texas in Jan. 1944, approximately 1 month before the onset of his present illness. There is no history of previous pulmonary disease or known contact with tuberculosis.

In April, 1944, shortly after arriving in North Africa, he had a recurrence of pain in his left chest lasting for 3 days accompanied by night sweats, weakness and a cough productive of small amounts of mucoid sputum. There was no hemoptysis. He lost 10 to 15 pounds in about 6 weeks.

On April 18, 1944, he was admitted to a station hospital in North Africa. Here a friction rub was heard over the left chest in the axillary line. His sedimentation rate was normal; sputum examination revealed no tubercle bacilli or fungi. The plasma proteins were normal. The blood count was normal and the urine revealed no abnormal findings. A Roentgen ray of the chest showed "a diffuse mottling throughout both lung fields that had the appearance of pulmonary

infiltration. The etiology may be fungus or tbc. in nature." (Fig. 1). During the 17 days spent in this hospital he complained of moderate pain in his left chest. His sputum was scanty, he ran a maximum temperature up to 99° the first 2 days only, and his general condition remained "excellent." On May 25, 1944, he was transferred to a general hospital with a diagnosis of probable coccidioidomycosis contracted in Texas.

suggests metastatic tumor in spite of the fact that the lesions are not sharply outlined or uniform in shape. A radiograph of the skull shows no cranial or intracranial abnormalities." A final Roentgen ray of the chest taken on June 9, 1944, showed "slight clearing of the patchy inflammatory process in both lungs since May 22. No cavities can be identified." (Fig. 2.)

On July 7, 1944, he was evacuated to the

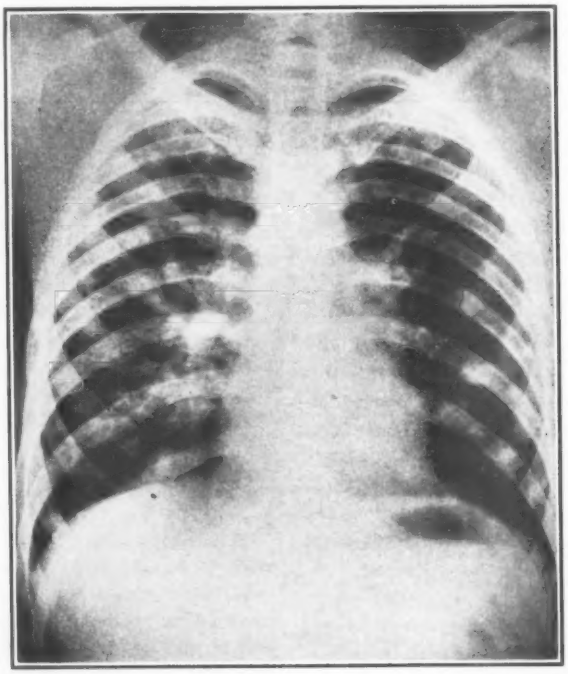

FIG. 1.—Roentgen ray of the chest, April 18, 1944, shows a diffuse mottling throughout both lung fields giving the appearance of "soft infiltrations." There is moderate enlargement of the hilar lymph nodes bilaterally.

Further studies were done to exclude a primary neoplasm with metastasis to the lungs. A complete G.I. series and intravenous pyelogram revealed no abnormalities. The sedimentation rates were normal, the blood counts and urinalyses were normal and repeated examination of the sputum revealed no tubercle bacilli or fungi. The radiologist reported that "Chest radiographs made elsewhere on April 19, and on May 7 and 22, show an extensive disseminated bilateral patchy pulmonary infiltrative process which seems to be progressive; the appearance is not characteristic, but rather

United States for further observation and treatment.

Physical examination on July 17, 1944, revealed a well developed and well nourished colored male who weighed 161¼ pounds (160 normal). He did not appear ill. The thorax was well developed and symmetrical and expansion was good. Harsh, rasping breath sounds were heard over the entire chest posteriorly. No friction rub was heard. The heart was normal, the blood pressure was 124/72. The skin was clear, the skeletal system appeared normal and

there was no adenopathy. The liver and spleen were not enlarged.

Laboratory Findings. Roentgen ray film of the chest shows infiltrations throughout both lung fields with little change as compared with the film of June 6, 1944 (Fig. 3).

Blood:

RBC	4,540,000
Hb	16.5 gm.
WBC	4700
Neut.	50
Lymph.	40
Mono.	9
Eos.	1
NPN	30.0 mg. per 100 ml.
Total protein	6.6 gm. per 100 ml.
Albumin	4.6 gm. per 100 ml.
Globulin	2.0 gm. per 100 ml.
A/G ratio	2.3

Sputum—Repeated 24-hour concentrates negative for tubercle bacilli; 3 successive gastric washings negative for tubercle bacilli; 3 sputum examinations and 3 gastric washings on July 27 and 31 and Aug. 1, revealed a species of fungus Imperfecta, Geotrichum.

Progress. This patient was given 15 gr. of sodium iodide intravenously daily and 20 drops of saturated solution of potassium iodide orally 3 times a day for the first week. The sodium iodide was then increased to 30 gr. and the saturated solution of potassium iodide increased to 120 drops a day. A total of 35 gm. of sodium iodide intravenously was given, following which he continued on saturated solution of potassium iodide until there was complete clearing of the pulmonary lesions. During treatment he showed a remarkable improvement radiologically and in a period of 3 months there was complete clearing of the pulmonary infiltrations (Fig. 4). Improvement was

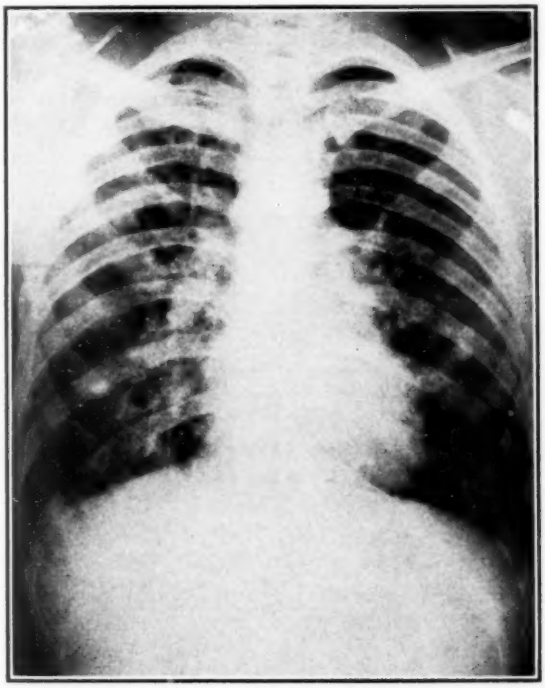

FIG. 2.—Roentgen ray of the chest June 6, 1944, shows slight clearing of the infiltration noted on April 18, 1944.

Sedimentation Rate—2mm/30 min. Westergren.

Urinalysis—negative.

Coccidioidin Intradermal Test—Negative in dil. 1–1000, 1–100, 1–10.

Tuberculin Test—Negative in dil. 1–100,000, 1–10,000.

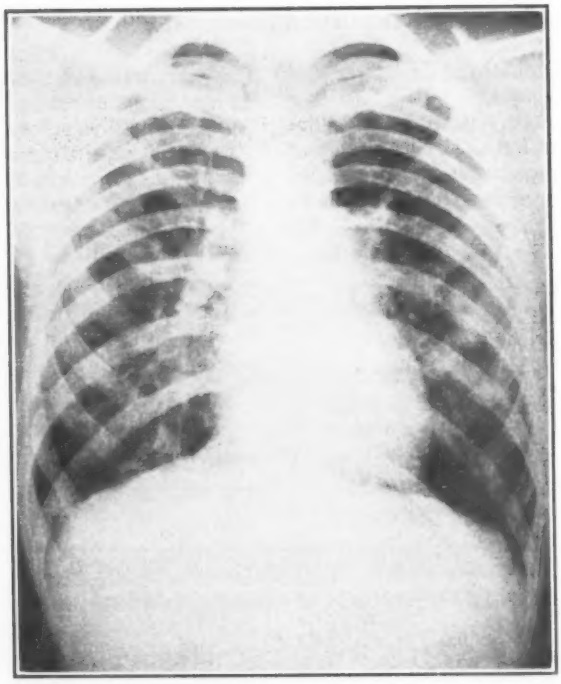

FIG. 3.—Roentgen ray of the chest, July 17, 1944, shows little change in the appearance of the lungs as compared with film of June 6, 1944.

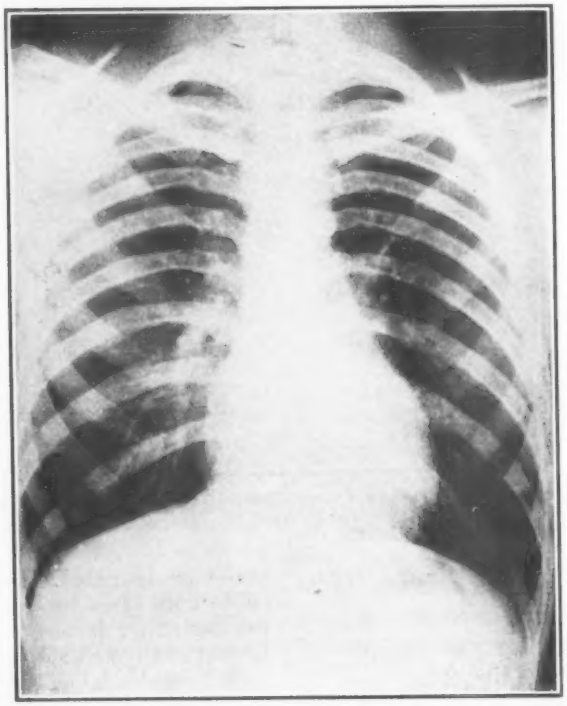

FIG. 4.—Roentgen ray of the chest, Nov. 11, 1944, shows complete clearing of the pulmonary infiltrations, decrease in size of the hilar lymph nodes, and slight increase in the bronchial markings extending out from the right hilar region.

noticed after 1 week of therapy and the sputum became negative for fungi during the first week of treatment. He regained the 10 pounds he lost, his cough disappeared, and his general condition became excellent. At the time of this writing, he is taking part in Group III reconditioning program and will be sent back to duty of a limited nature.

Discussion. The increased frequency with which mycotic disease of the lungs is being reported in the literature probably indicates a sharpening of diagnostic acumen rather than an actual rise in the incidents of mycotic disease in general. Frequently mycotic disease is being salvaged from the waste basket of nonspecific pulmonary disease, atypical pneumonitis, and unproved pulmonary tuberculosis.

FIG. 5.—Four day old colonies from sputum on Sabouraud's agar at room temperature.

It is true that yeast organisms may "contaminate" the respiratory tract as non-pathogenic organisms, and also sputum specimens in the laboratory. However, it is also recognized that they may act as both primary and secondary pathogenic invaders, more often the latter.

Campbell,[2] following a study of a good number of yeast infections of the lung, believes that from a practical standpoint there seems to be sufficient similarity in lesions produced to warrant discussion of the histological tissue findings, Roentgen findings, and sequelæ of all yeast infections as though they were a single entity and any variation one only of degree of variance and not necessarily characteristic for the species. However, we believe that identification of the species is of importance in selecting therapeutic measures.

From the standpoint of symptomatology and clinical findings there is nothing significant in regard to geotrichum infection to differentiate it from other types of mycotic disease. Peterson[7] recently justifiably stated that "any lesion having the appearance of tuberculosis in which no tubercle bacilli can be found, should be studied carefully to rule out a fungus infection. In patients in whom no definite organisms can be isolated, a therapeutic test with iodine may be instituted and the final diagnosis based on the response." A white mucoid sputum containing grayish flakes, having an yeast-like odor should suggest a fungus disease.[9] Further identification of the type will have to be made on the basis of cultural characteristics.

Macroscopically the organism is grown

on Sabouraud's media—the colony is developed in 24 to 48 hours at room temperature. They are small, white, fuzzy and adherent to the agar (Fig. 5). Giant colonies develop in 10 days and show a radiating appearance with abundant coremia (Fig. 6). The strain isolated repeatedly from the sputum of our patient liquefied both gelatin and litmus milk. Acid was formed from dextrose, maltose, sucrose and lactose. This strain of geotrichum shows some of the characteristics of G. Versiforme (Dodge[4]).*

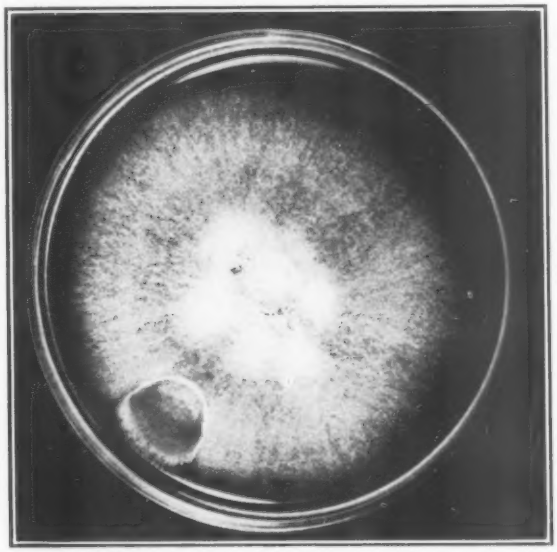

FIG. 6.—Ten day old giant colony on Sabouraud's agar at room temperature.

Microscopically the mycelium is septate and displays both arthrospores and chlasmydospores. The arthrospores are very abundant in young (2 days) preparations and appear as bacillary and coccoidal forms at 440 diameter magnification. Baker, *et al*[1] in 1943 considered geotrichum as a variety of coccidioides. However, culture of several coccidioides immitis studied here and those described by Dodge[4] did not show the conidia or arthrospores so dispersed as to resemble bacterial forms characteristic of geotrichum.

It is highly important that repeated examination of the sputum for tubercle bacilli be performed before tuberculosis is eliminated. Not infrequently tuberculosis may coëxist with fungus disease. In our case, both the tuberculin and the coccidioidin skin tests were negative.

Recently geotrichum was isolated from the sputum of 2 other cases of chronic respiratory tract disease. In 1 case bronchiectasis and asthma were suspected; both were ruled out on the basis of normal lipiodol studies and inconclusive skin tests for protein sensitivity. However, a chronic bronchitis was present which, from the history, evidently followed an atypical pulmonary infiltration which he developed while in North Africa. It is interesting that recent skin tests revealed that he was sensitive to several molds including aspergillus, monilia, and penicillium. The second case also was one of chronic

* The Army Medical School and The Fungus Laboratories of Duke University confirmed the diagnosis of geotrichum. A recent communication from Norman F. Conant, Ph.D. of the Department of Medicine, Duke University, stated that the fungus was identified as *geotrichum sp.*, a generic identification only, since the exact species could not be identified.

bronchitis with asthmatic symptoms who showed nothing definite on skin testing, but who repeatedly showed geotrichum in his sputum. Radiologic examination of both patients showed no evidence of pulmonary parenchymal involvement.

Conclusion and Summary. Geotrichum has been isolated repeatedly from the sputum of 3 patients, 1 of whom presented symptoms and radiologic findings of chronic bronchopulmonary disease; the other 2 presented symptoms of chronic bronchitis and asthma with no evidence of pulmonary infiltration. The clinical findings and the radiologic appearance of the lesions are not specific for geotrichum alone, but are not unlike those of mycotic disease in general. Furthermore, the symptoms and clinical findings may readily be confused with tuberculosis and atypical pneumonitis. An accurate diagnosis is important from the standpoint of treatment and disposition of the patient. Geotrichum apparently responds well to massive iodide therapy with complete clearing of the lesions and disappearance of the bronchopulmonary symptoms.

It is realized the spontaneous resolution of mycotic pulmonary lesions not infrequently occurs in mild infections. Spontaneous improvement occurred in the case herein reported. However, we have every reason to believe that clinical recovery and disappearance of the fungus from the sputum was expedited by the iodide therapy. It is true that secondary infection coëxists with or complicates fungus disease altering the clinical picture and retarding the recovery of the patient. Fortunately, our patient has no apparent secondary infection to interfere with recovery.

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THE PENETRATION OF ANTIBACTERIAL SUBSTANCES INTO ISCHEMIC, INFLAMMATORY TISSUE

WITH A CONSIDERATION OF THEIR USE IN SHOCK OF BACTERIAL ORIGIN*†

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THE incidence of war wounds involving trauma to muscles stimulated renewed interest in the mechanism of the shock syndrome which often follows such injury. In an endeavor to ascertain the causes of shock due to muscle injury alone, a method was devised in this laboratory[15] which made it possible to produce shock regularly in dogs (50 out of 52 animals) by crushing a known amount of muscle. Bleeding was minimal. In this procedure, the quadriceps femoris of anesthetized animals was removed from one hind limb under sterile precautions, ground in a sterile mortar and replaced in its original bed. After operation, the limb became swollen and at the end of 24 hours, the circulating blood volume was significantly reduced and the animal entered into a state of shock.

Of particular interest was the fact that local bacterial contamination occurred in each instance in spite of rigid aseptic technique. The crushed muscle at autopsy had a foul odor, gas was often present and smears revealed numerous bacteria of all types including *Clostridium welchii*. It was ascertained by actual measurement that the fluid shift to the traumatized extremity was not sufficient to account for the development of shock.[15] Furthermore, the application of plaster casts to the traumatized limb minimized local accumulation of fluid but did not prevent the onset of shock.[15] Debridement of the crushed muscle prevented shock when undertaken within 12 hours of the time of operation, but was ineffective when performed after 17 hours. In 50 animals treated with sulfamerazine, shock was prevented in each instance; there was less swelling of the traumatized limb and no significant reduction of the circulating blood volume. When the animals were sacrificed, the crushed muscle at autopsy appeared clean, was free of odor and few or no organisms were found on direct smear. The conclusion was drawn that shock in these experiments was due to systemic absorption of toxic products from the infected muscle tissue.

In subsequent experiments[9] it was found that sulfamerazine was completely effective in preventing shock if given up to 6 hours after the muscle-crushing operation, slightly beneficial if administered at the end of 17 hours, but without value if withheld for 24 hours. When this drug was applied locally to the crushed muscle, and none administered in any other way, the protective effect was variable, since shock occurred in 2 of 11 animals treated in this manner. In further experiments with the parenteral administration of this agent, it was found that the traumatized muscle contained a much higher concentration of the drug than did the blood or uninjured muscle of the same animal.

* The work described in this paper was done under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the Mount Zion Hospital.

† The bacteriologic aspect of this project was conducted in collaboration with Dr. Karl F. Meyer, Hooper Foundation, San Francisco.

The experiments described in previous reports from this laboratory[9,15] are concerned chiefly with the treatment of toxic (infectious) shock with sulfamerazine. Because of the demonstrated potency of penicillin as a bacteriostatic agent, it was considered important to test the action of this drug in this type of shock. It is the purpose of this communication (*a*) to compare the effectiveness of penicillin and sulfamerazine in preventing shock due to muscle crushing, when these agents are given locally or systemically, and (*b*) to compare the respective ability of penicillin and sulfonamides to penetrate ischemic, crushed muscle, when these agents are administered intravenously, and (*c*) to discuss the treatment of shock resulting from wounds involving extensive damage to tissues.

Methods. *Technique of Muscle-crushing Procedure.* The quadriceps femoris of dogs anesthetized with sodium pentobarbital was removed from one hind limb under sterile technique. The muscle was cut into fine pieces, ground in a sterile mortar and then replaced in its original bed, after which the incision was closed. The amount of muscle crushed corresponded to 3 to 5 gm. per kg. body weight.

Plasma Volumes. Estimations were made according to the method of Gregersen and Gibson,[10,13] employing the blue dye T–1824. The concentration of dye in plasma was determined by means of the Evelyn photoelectric colorimeter.[5,11]

Penicillin Determinations. The methods of determining the concentration of penicillin in the blood and muscle will be described under the appropriate experiment.

EXPERIMENT 1. *The Parenteral Administration of Penicillin.* The following experiment was performed to ascertain the effectiveness of penicillin, systemically administered, in preventing and counteracting shock due to muscle crushing. The muscle crushing procedure was performed on 8 dogs, weighing 9.1 to 12.7 kg. A series of intramuscular injections of sodium penicillin (5000 Oxford units) was begun in each animal within 15 minutes after operation and continued every 3 hours for 36 hours.

Of the 8 dogs, all but 2 developed shock within 12 to 24 hours after operation, and all but 1 recovered. The animals were sacrificed when it became evident that satisfactory recovery had taken place. The wounds at autopsy in all but the fatal case appeared clean and free of odor, although cultures of the crushed muscle yielded several types of bacteria, including *Cl. welchii* and *Streptococcus viridans*. A decrease in plamsa volume was observed in each of the 6 dogs which developed shock, the greatest reduction having been noted in the animal that died, in which instance the value fell to 41% of the normal. The traumatized leg in the fatal case was greatly swollen and at autopsy the wound was filled with gas, hemolyzed fluid was present and culture showed gross bacterial invasion.

In a previous paper,[9] it was reported that all but 2 of 52 dogs subjected to the muscle-crushing procedure developed shock. If the results of treatment with penicillin given systemically are compared with these control findings, it is evident that penicillin was of some benefit, since shock was prevented in 2 of 8 treated animals. When, however, the results with penicillin and sulfamerazine are compared, it is clearly established that penicillin, systemically administered, is inferior to sulfamerazine, which is completely effective in preventing this type of shock.[9]

EXPERIMENT 2. *The Local Use of Penicillin.* The following experiment was performed to ascertain the ability of penicillin, locally administered, to counteract shock due to muscle crushing. Eight dogs were subjected to the muscle-crushing procedure. Before reinsertion, the crushed muscle was thoroughly mixed with an isotonic saline solution of sodium penicillin containing 500 units per cc. No other treatment was administered. The animals were observed for a period of 6 to 8 days and at no time exhibited symptoms of shock. The traumatized legs showed slight to moderate swelling and at autopsy the wounds appeared clean and free of odor.

From this experiment, it is concluded that the local instillation of penicillin is completely effective in preventing this type of shock. In a parallel study,[9] it was found that the local application of sulfamerazine prevented shock in 9 of the 11 dogs tested. The somewhat less successful results with the local use of sulfamerazine can be explained by the fact that penicillin has been

found to be superior to the sulfonamides in this type of infection.[1,14]

EXPERIMENT 3. *Concentration of Penicillin in Blood and Crushed Muscle.* The purpose of the following experiment and the succeeding one was to attempt to explain the results of systemic treatment with penicillin and sulfamerazine by the respective ability of these substances to penetrate and accumulate in the ischemic, crushed muscle. A single, large intravenous dose of sodium penicillin (5000 units per kg. of body weight) was given to 6 dogs immediately after subjecting them to the muscle-crushing operation. Samples of blood and crushed muscle were taken 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, and 5 hours, respectively, after the drug was injected, and the amount of penicillin in each sample determined.

The method of analysis was as follows: The concentration of penicillin in the blood was determined: (*a*) by comparing the inhibitory effects of 0.1 cc. of blood sample with those of 0.1 cc. of known dilutions of penicillin upon colonies of *Staphylococcus aureus* (Heatley) in agar plates,[6] and (*b*) by titrations in broth,[16] using *Strep. hemolyticus* (New York 5 strain) with 1% sheep erythrocytes in beef heart broth media, taking prevention of hemolysis as an end-point. For the titrations in broth, penicillin solutions of 10 units per cc. and 1 unit per cc. were run concurrently as controls. Muscle samples were weighed, cut up into small pieces and ground with fine, washed and fused sea sand, with or without broth. The resulting pastes or masses were centrifuged at 24,000 to 28,000 r.p.m. for 10 to 15 minutes. The supernatant liquids were assayed for penicillin potency directly by the agar cup method.[6] Broth titrations by Rammelkamp's method[16] were run with samples which were sterilized by filtration. Results were adjusted by dilution factors when broth was added in the grinding process.

It was found that the blood concentration of penicillin fell rapidly after attaining an initially high level (Fig. 1). The curves were similar to those of other investigators.[3,17] At the end of 1 hour, the average concentration of penicillin in the blood was 6.1 units per cc., but the concentration in the muscle was only 0.6 unit per gm. The highest concentration in any of the individual muscle samples was 1.2 units per gm. and was reached 2 hours after the drug was administered (Fig. 1).

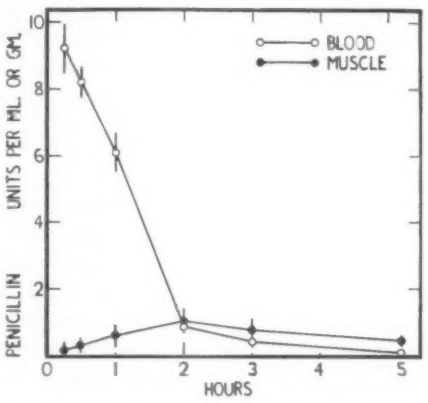

FIG. 1.—Average concentration of penicillin in blood and crushed muscle in 6 dogs after a single, large, intravenous injection (5000 units per kg. body weight). The length of the vertical lines at each point is equal to 2 × the standard deviation of a single observation. The concentration of penicillin in the blood fell rapidly after the initial high level. The maximum concentration in the crushed muscle (1.2 units per gm.) was attained 2 hours after injection.

The failure to achieve a satisfactory concentration of penicillin in the traumatized area after injection in amounts yielding an adequate blood level explains the inability of this agent to be fully effective after parenteral administration. This experiment does not exclude the possibility that if a high blood level of penicillin could be maintained over an adequate period of time, the drug would eventually accumulate in the ischemic, crushed muscle in bacteriostatically effective amounts.

EXPERIMENT 4. *Concentration of Sulfamerazine in Blood and Crushed Muscle.* Four dogs were subjected to the muscle-crushing procedure. Immediately after operation, each animal received a single, large intravenous dose of sodium sulfamerazine (0.2 gm. per kg. of body weight). No subsequent medication was given. At various intervals after the administration of the drug (15 minutes, 6 hours, 24 hours, 54 hours, 78 hours), samples of blood and crushed muscle were taken and the concentration of sulfamerazine in each was determined by a modification of Bratton and Marshall's method.[2]

It was found that the blood level was

highest at the first determination made 15 minutes after injection, and steadily declined thereafter until none or only a trace was found at the end of 78 hours. The concentration in the crushed muscle was very low at the end of the 15 minute period, rose gradually and reached its peak at the end of 24 hours, at which time the concentration approximated the maximum blood level (34.4 mg. per 100 cc.). After 24 hours, the concentration curve declined slowly and at the end of 78 hours the muscle still contained between 1.5 and 2 mg. per 100 gm. (Fig. 2). It is of interest to note that sulfamerazine attained levels in the muscle as high or higher than in the blood, while penicillin did not.

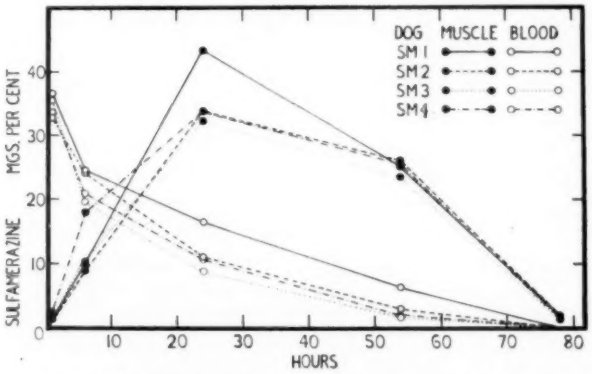

FIG. 2.—Concentration of sulfamerazine in blood and crushed muscle in 4 dogs after a single, large intravenous injection (0.2 gm. per kg. body weight). The blood level was highest at the first determination, 15 minutes after injection, and declined steadily until none or only a trace was found at 78 hours. The concentration in the crushed muscle was very low at the 15 minute period, reached its peak at the end of 24 hours, and then decreased gradually. At the 78 hour determination, the muscle still contained between 1.5 and 2 mg. of sulfamerazine per 100 gm.

According to Fox, sulfonamides circulate in body fluids partly in unionized molecular form, and partly in ionized form. The undissociated form diffuses freely in and out of the tissues, but the ionized sodium salt after entering the traumatized area is bound there like other sodium salts (Fox and Keston[7]). The latter fact helps to explain why sodium sulfamerazine, which is highly ionized at the pH of body fluids (68.5% at pH 7.4[12]), is retained in injured tissues in such large concentrations.

EXPERIMENT 5. *Concentration of Sulfanilamide in Blood and Crushed Muscle.* Inasmuch as sulfanilamide in solution is almost completely undissociated (99.9% at pH 7.4[12]), it was anticipated that after intravenous injection a smaller proportion would penetrate the traumatized muscle than in the instance of sulfamerazine. Accordingly, the following experiment was performed. Four dogs were subjected to the muscle-crushing procedure. Immediately after operation, each animal received a single, large, intravenous dose of sulfanilamide (0.2 gm. per kg. of body weight). At various intervals after injection (15 minutes, 6 hours, 24 hours, 54 hours, and 78 hours), samples of blood and crushed muscle were taken and the concentration of sulfanilamide in each sample determined.

It was found that the blood level was highest at the determination made 15 minutes after injection (22.5 mg. per 100 cc.), and that the concentration curve declined steadily until only traces of the drug were found at the end of 78 hours. The crushed muscle contained only a trace at the 15 minute period. At the end of 6 hours the concentration reached its peak (16.4 mg. per 100 gm.), then gradually decreased until only a trace was present at 78 hours (Fig. 3).

These results indicate that after intravenous injection a smaller concentration of sulfanilamide accumulates in the crushed muscle than in the case of sulfamerazine. This finding confirms Fox's observation[8] that a drug which is undissociated in the blood would accumulate in injured tissues to a lesser extent than one which is extensively ionized.

Discussion. In previous reports from this laboratory,[9,15] it was shown in dogs that sodium sulfamerazine, systemically administered, prevented shock produced by a crushing injury to muscle. In these experiments it was found that shock was a direct result of bacterial infection in the traumatized tissue, and the effectiveness of sulfamerazine was attributable to its antibacterial activity. The local application of sodium sulfamerzaine was also effective in preventing shock, but not nearly so uniformly as after systemic administration.

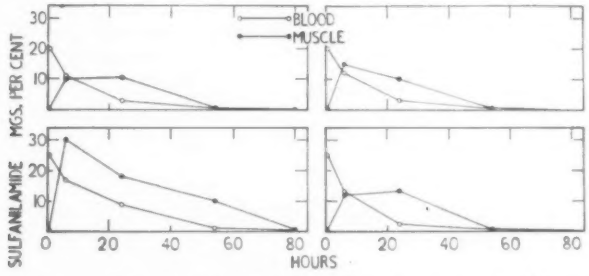

FIG. 3.—Concentration of sulfanilamide in blood and crushed muscle in 4 dogs after a single, large, intravenous injection (0.2 gm. per kg. body weight). The blood level was highest at the first determination 15 minutes after injection, and declined steadily until only traces of the drug were found at the end of 78 hours. The crushed muscle contained only a trace at the end of 15 minutes. The concentration was at the maximum after 6 hours, and declined gradually. At 78 hours, only a trace was present.

In the experiments reported in this paper, sodium penicillin, applied locally to the crushed muscle, was found to be completely effective in preventing the onset of shock, but beneficial only to a variable extent upon parenteral administration.

In an endeavor to explain the results after systemic administration of these drugs, a study was made of their respective penetrability into traumatized, ischemic tissue. Assays of these antibacterial agents in the crushed muscle after intravenous injection, showed that sulfamerazine is readily diffused into the injured tissue in bacteriostatically effective concentration, whereas penicillin is accumulated in mildly inhibitory levels only. Prolonged high levels in the blood are easily attained with sulfamerazine, a fact which appears to be explained by its low excretion rate and its high protein-binding power (80% at pH 7.4[12]). The achievement of sustained high plasma concentrations of this drug helps to explain its prolonged diffusion into the injured area.

Fox and Keston[7] employed a radioactive isotope of sodium (Na^{24}) to trace the movement of this element in mice subjected to severe injury by burning or tourniquet trauma. Experiments showed that traumatized tissues attracted and retained sodium ions. The amount of sodium retention far exceeded the gain in water (edema), a fact which indicates that additional sodium accumulated in the intracellular compartment. Administration of isotonic saline solution containing radiosodium was followed by nearly complete retention of sodium in the injured animals and by excretion of sodium in normal controls.

At the hydrogen ion concentration of the blood, sulfamerazine is ionized to a considerable extent (68.5%).[12] The importance of this observation lies in the fact that only the ionized portions of the sulfonamide salts appear to exert antibacterial action.[8] The undissociated form is distributed with body water and diffuses freely in and out of the tissues like other unionized molecules, *e. g.*, urea and glucose. The ionized form of sulfamerazine upon entering the traumatized area is bound there like other sodium salts, and this fact explains the high concentration of this

chemical in ischemic, crushed muscle after systemic administration.

Sulfanilamide at the hydrogen ion concentration of body fluids is ionized only to the extent of 0.1% and is bound to plasma protein to the extent of 20%,[4] and as the results of assay show does not accumulate in the blood and traumatized muscle in as high concentration as sulfamerazine.

The low concentration of penicillin attained in traumatized tissue would serve to explain why the systemic administration of this agent is not entirely effective in counteracting shock due to muscle crushing.

The penetration and accumulation of various antibacterial agents into traumatized, ischemic and infected areas is not only of theoretical interest, but is also of obvious practical importance, for this type of injury is a fairly common occurrence not only on the battlefield but also in civilian life.

In treating wounds with antibacterial agents, it is necessary to take several factors into consideration. Drugs should be chosen according to (*a*) their specificity for the type of bacteria present, and (*b*) the route of administration, which in turn depends in part upon the blood supply to the wound.

For local therapy, penicillin appears to be superior to sulfamerazine. The local application of penicillin without additional medication prevented shock, whereas the topical administration of sulfamerazine was not entirely effective.

If local therapy is not possible or supplementary systemic treatment seems advisable, the sulfonamide of choice would be one which possesses great bacteriostatic potency, ready diffusibility, and a high coefficient of retention in the blood and injured tissue. Sodium sulfamerazine, a highly ionized salt, was found to fulfill these requirements more satisfactorily than sulfanilamide, an almost wholly undissociated substance. From these observations, it is suggested that an injury involving the crushing of muscle should be treated by administering systemically an ionized sulfonamide such as sodium sulfamerazine and performing debridement, which as previously stated has been shown to be effective in preventing shock. If in addition the use of a local antibacterial agent is considered desirable, penicillin rather than a sulfonamide would appear to be the drug of choice.

Summary and Conclusions. Muscle crushing experiments were performed in dogs. Shock developed as a result of systemic absorption of toxins from the muscle tissue which became infected in spite of rigid surgical technique. The treatment of shock induced in this manner was investigated by the use of antibacterial agents.

1. The instillation of sodium penicillin at the site of injury was found to be completely effective and superior to the local application of sodium sulfamerazine.

2. The systemic administration of sodium penicillin was shown to be of benefit, but not so uniformly effective as the systemic use of sodium sulfamerazine. This fact is in keeping with the demonstration that given in the manner described penicillin does not penetrate ischemic muscle tissue in amounts that are uniformly bacteriostatic, whereas sodium sulfamerazine does.

3. Sodium sulfamerazine, intravenously injected, penetrates into ischemic, crushed muscle tissue in larger amounts than sulfanilamide, similarly applied. The difference in the penetrability of these substances is explained by their relative degree of ionization, sodium sulfamerazine being highly ionized at the hydrogen ion concentration of body fluids and sulfanilamide being almost completely undissociated.

4. The treatment of crushing muscle injuries suggested by these observations is immediate systemic injection of an ionized sulfonamide, like sodium sulfamerazine, followed by complete debridement of the wound. These procedures can be supplemented by the local application of an antibacterial agent, and for this purpose sodium penicillin is superior to sodium sulfamerazine.

The authors desire to thank Dr. Charles L. Fox and Dr. Ben Sacks for their valuable aid in the preparation of this paper.

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ACUTE PLEURISY AS A DEHYDRATION PHENOMENON IN DIABETIC PRECOMA

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Acute dry pleurisy in a known diabetic should be regarded as an infectious pleuritis until proven otherwise. The incidence of respiratory infections including pulmonary tuberculosis is apparently higher in a diabetic person than in the general population.[1,5] Such infections as a cause of pleurisy in any diabetic patient should be considered before attempting to make other etiologic diagnoses.

We have studied 5 diabetic patients who entered the hospital with uncontrolled diabetes and coëxistent acute dry pleurisy. The marked loss of extracellular fluid which occurs in the precoma phase appears to be the chief cause of this phenomenon.

Case Reports. Case 1. N. G., a 30 year old white male, entered Vanderbilt University Hospital in June, 1944 with a 6 year history of diabetes mellitus. He had been taking 36 units of protamine zinc insulin daily, but had lapsed in his diet and failed to take his insulin regularly.

Six days before admission he grew weak and developed anorexia. Later he noted polyuria and polydipsia. On the day of entry he noted pain in his epigastrium and along both costal margins anteriorly. This pain was greatly aggravated by respiration which "cut off" his breath, and it tended to move to the side on which he would lie. There was no history of cough, hemoptysis, dyspnea, or previous chest pain.

Examination revealed an undernourished, acutely ill man whose breathing was deep and rapid and whose breath smelled of acetone. His temperature was normal. The skin and mucous membranes were very dry. The heart and lungs were normal except for bilateral friction rubs heard low in both axillæ. The abdomen was moderately rigid with slight epigastric tenderness.

Laboratory studies revealed glycosuria, acetonuria, a red blood cell count of 6.11 million per cmm., 16.5 gm. of hemoglobin per 100 cc., and a white blood cell count of 25,400 per cmm. The blood sugar was 480 mg. %. Blood CO_2 combining power was not determined on admission. Within 14 hours after admission the patient had received 6600 cc. of normal saline solution and 140 units of regular insulin, and was fairly well hydrated. He felt well and no longer complained of abdominal or chest pain. The friction rubs had disappeared. A Roentgen ray examination of the chest showed no abnormalities. An old tuberculin skin test of unknown dilution was positive. The patient was discharged asymptomatic in 2 weeks well regulated on 64 units of globin insulin daily. Nine months later there was no evidence of chest disease.

Case 2. R. J., a 20 year old college student known to have diabetes for 4 years, entered Vanderbilt University Hospital in August, 1944 in severe diabetic acidosis. He always had been poorly regulated and had been haphazard in his diet and in taking his insulin.

During the week preceding admission polyphagia, polyuria, and polydipsia began. Several urine specimens showed large amounts of sugar. The day before admission he was nauseated, did not eat and did not take his insulin. He became drowsy, and his respirations increased in depth and frequency. He developed severe anterior chest pain which was aggravated by breathing. There were no other respiratory symptoms. A Roentgen ray examination of his chest 1 year previously was negative.

Physical examination revealed an afebrile, but acutely ill, young man, complaining of

severe pain in his chest. There was marked Kussmaul breathing. He was drowsy but could be aroused. His skin was cool and dry, and his face was flushed. Mouth membranes were very dry. Friction rubs were heard low in both axillæ. No râles were heard, and the heart was normal. The blood pressure was 140/80 on entry, but fell to 110/65 within an hour. The abdomen was not tender. The remainder of the examination was not remarkable.

The laboratory examinations included a red blood cell count of 4.89 million per cmm., a hemoglobin of 15 gm. per 100 cc., a white blood cell count of 19,150 per cmm., and urine showing glucose and acetone. The urinary sediment was normal. The blood sugar was more than 400 mg. per 100 cc. Blood CO_2 combining power was not determined.

He was given 5400 cc. of normal saline solution subcutaneously on the night of admission and also 140 units of regular insulin. The stomach was aspirated and lavaged with 5% sodium bicarbonate solution. He improved rapidly on this régime. The following morning, 16 hours after admission, the pleuritic pain and friction rubs had disappeared. A Roentgen ray examination of the chest on this day was negative. An old tuberculin skin test using 0.001 mg., was negative. He was discharged on 36 units of regular insulin and 24 units of protamine zinc insulin. One month later he felt well and had no symptoms of pleuro-pulmonary disease.

Case 3. C. H., a 21 year old white man, re-entered Vanderbilt University Hospital in June, 1944, with diabetes mellitus of 15 months duration. It had started after an attack of measles with increasing weakness, loss of weight, polydipsia, polyuria, polyphagia and nocturia. He had been regulated on a diet and 40 units of protamine zinc insulin daily.

On the day before admission he felt tired, lost his appetite and noted a slight cold with a nasal discharge. On the morning of admission he vomited twice and soon afterwards was seized suddenly with severe, sharp pain in both costo-vertebral angles which was made much worse by inspiration. The pain was knife-like and cut off his breath; holding his breath made it cease. He had no chills, fever, cough, hemoptysis, dyspnea or abdominal pain. There was no history of previous chest pain.

Physical examination revealed a somnolent young man in acute respiratory distress complaining of chest pain with each breath. His temperature was normal. There was a strong odor of acetone on his breath, and his breathing was of the Kussmaul type. His skin and oral mucosa were very dry. At both lung bases in the posterior axillary lines scratchy pleural friction rubs were heard. A similar rub was also heard just to the right of the sternum in the fourth interspace anteriorly. The examination of the heart and lungs was otherwise negative. The abdomen was normal.

Laboratory findings included glycosuria and acetonuria. There were 6.3 million red blood cells and 32,500 white blood cells per cmm., and a hemoglobin of 17 gm. per 100 cc. of blood. The blood sugar was 400 mg. per 100 cc. and the blood CO_2 combining power was 30 vol. % 12 hours after admission.

In a period of 15 hours the patient was given 8100 cc. of normal saline solution and 200 units of regular insulin. At this time he was fairly well rehydrated and no longer had chest pain nor audible pleuritic friction rubs. A chest film showed no evidence of pulmonary or pleural disease. Intracutaneous old tuberculin, 0.1 mg., gave a positive reaction. After 2 afebrile weeks he was discharged asymptomatic and well regulated on a diet and 56 units of globin insulin daily. He has not been seen subsequently.

Case 4. L. I., a 17 year old white female with diabetes of 9 years duration, was admitted to Vanderbilt University Hospital in December, 1940 in diabetic coma with dehydration and acidosis. Symptoms had started 2 days previously when she developed a gastro-intestinal upset coincident with her menstrual period. She became nauseated, did not eat well, omitted 2 doses of insulin, and started to vomit. She became drowsy, developed deep and rapid breathing, and then lapsed into coma a few hours before admission. There were no other respiratory symptoms. No chest pain or recent infections were admitted by the patient later. Previously she had been well controlled on 72 units of protamine zinc insulin and 36 units of regular insulin daily.

On physical examination she was an obese, comatose, very dehydrated young white woman with deep respirations and the odor

of acetone on her breath. At the right lung base posteriorly there was a short friction rub and showers of fine râles on deep inspiration. The remainder of the examination was not remarkable.

Laboratory studies included glycosuria and acetonuria, a blood sugar of 456 mg. per 100 cc. and a blood CO_2 combining power of 16 vol. %. She had a leukocytosis of 21,000, but later the same day and subsequently her white blood cell count varied between 5800 and 7400. A Roentgen ray examination of the chest was negative.

The râles and friction rub disappeared within a few hours after parenteral fluids and insulin were administered. She received 140 units of regular insulin and 60 units of protamine zinc insulin plus 7000 cc. of fluids in the form of saline solution and glucose in saline solution in the first 24 hours, and regained consciousness. She has been followed for more than 3 years with several Roentgen ray examinations of the chest. There has been no evidence of pulmonary tuberculosis. A tuberculin test was not done.

Case 5. M. M., a 16 year old white girl known to have diabetes for 4 years, entered Vanderbilt University Hospital in January, 1935, complaining of pain in the "side" and drowsiness.

Four days before this admission she developed a cold after exposure to snow and sleet. A little later she noted pain on breathing in the right lower chest which persisted to the time of admission. There was a slight non-productive cough but no chills or fever.

Physical examination showed a drowsy, dehydrated girl with Kussmaul respirations. A friction rub with a few showers of fine râles was present at the right lung base posteriorly; the left chest was clear. She was afebrile.

She had sugar and acetone in her urine. Her blood sugar was 356 mg. per 100 cc., and the blood CO_2 combining power was 15 vol. %. A chest film showed diminished penetration over the left base suggestive of early pneumonia, but the right chest where the abnormal physical findings were noted was entirely clear.

The pleural friction rub disappeared during the first 24 hours of hospitalization after the administration of fluids and insulin. She was followed with numerous chest Roentgen ray examinations after the discharge, and has never developed roentgenologic evidence of pulmonary tuberculosis. She died in 1944 of diabetic coma, staphylococcus septicemia and staphylococcic abscesses of the kidney.

Comment. In all the cases described, the pleuritic pain appeared in known diabetic patients whose disease was out of control. On admission to the hospital, 1 patient was comatose, but the others were drowsy or in the so-called "precoma" state. All showed much dehydration with acidosis and hyperglycemia. In each case the pleural friction rubs were heard by one or more observers.

In the first 2 patients failure to take insulin regularly and to adhere to diets was apparently the cause of the ketosis. The third patient had a cold with rhinitis on the day before admission without other respiratory symptoms. It was thought that this alone could hardly account for his being out of control a day later. The fourth patient presented a more complicated picture in that a gastro-intestinal upset, lapse in diet, failure to take insulin, and a menstrual period occurred at about the same time. This was the only patient to enter the hospital actually unconscious. Later no history of a previous respiratory infection could be elicited. The râles and the friction rub disappeared within a few hours. The râles could have been atelectatic as a postural manifestation following prolonged immobilization of the body in a supine position. After the administration of fluids the leukocyte count quickly dropped to normal, which would also be against an infectious cause of the râles. The fifth patient only gave a definite history of a previous respiratory infection with later development of right pleuritic pain and cough. She had no fever. The friction rub and showers of râles on inspiration were heard at the right base posteriorly. However, the Roentgen ray changes suggestive of early pneumonia were at the left base where there were no abnormal physical findings. If such it was, the left-sided pneumonitis may have helped to precipitate the precomatose state. It was impossible to say that her

acute pleurisy was not infectious in origin, but its rapid disappearance following administration of fluid and insulin was a point in favor of a non-infectious origin.

While at the time of admission it was thought that a respiratory infection was responsible for the acute pleurisy in each case, the clinical courses convinced us that this probably was not true. All patients remained afebrile, and definite evidence of pneumonitis as the cause of pleurisy was lacking. We realize that friction rubs tend to be transient in nature. However, in our series we feel that it was predominantly rehydration which resulted in their rapid and permanent disappearance. All patients were young adults in the age group for tuberculosis with pleurisy and effusion. None of these 5 patients subsequently developed a pleural effusion and in none was there any evidence of active pleuropulmonary disease at later dates.

Regardless of the causes of their diabetes being out of control, the patients were clinically similar in that all were very dehydrated. Within hours after adequate doses of insulin and isotonic saline solution, the patients became more or less fully rehydrated. Coincidentally both the chest pain and the pleural rubs disappeared. The leukocytosis which most showed on admission rapidly disappeared. Subsequently the hyperglycemia and acidosis were controlled within 1 to several days. Chest films failed to show any pulmonary or pleural abnormalities. It is to be emphasized that none of the patients received specific chemotherapy such as sulfonamides.

Discussion. Impending diabetic coma is due to marked changes in the chemical and fluid equilibria of the body and is usually accompanied by many well-known symptoms. Among them may be excessive urine output, deep and rapid respirations, drowsiness, and often nausea, vomiting and abdominal pain. The cause of the abdominal pain has never been fully explained, but Walker[7] believes that "gastric tetany" from chloride loss due to vomiting and diuresis may be responsible. There has been nothing reported in the medical literature concerning acute pleurisy in the diabetic precoma state. Marble[4] states that in a diabetic "any occurrence out of the ordinary should arouse suspicion, and one should instantly investigate any of the following symptoms: headache, anorexia, restlessness, weakness, listlessness, nausea, vomiting, drowsiness, and painful, rapid or deep breathing." There is no elaboration upon the symptom of painful breathing, nor is it stated whether or not it is pleuritic in nature.

Closely linked with changes in the electrolyte balance of the body fluids in diabetes is dehydration. This becomes manifest not only in the circulating fluid but also in the tissues themselves. According to Starling[6] normally the surfaces of the pleura are kept moist by the secretion of lymph onto their surfaces. It is conceivable that dryness of the pleura could be the main factor in the production of the acute pleurisy seen in these patients.

As polyuria, vomiting and therefore dehydration progress, the blood volume diminishes as reserves of tissue fluids are depleted. With further changes in the electrolyte pattern occurring, the patient may eventually present the picture of peripheral vascular collapse and shock. The blood pressure may be low, the skin may be cool and dry, and the usual moisture of the oral mucosa may be absent. In our patients we believe that the normal amount of lubricating pleural fluid became decreased or absent. Nondiabetic patients that we have seen who were dehydrated because of other conditions such as acute infections or pyloric obstruction did not exhibit friction rubs. The observation, that in the process of being rehydrated by parenteral fluid administration all of our patients soon recovered and the pleural manifestations quickly disappeared, would tend to support our hypothesis.

The location of the friction rubs was quite uniform—low in the chest in the axillary lines where motion between the

visceral and parietal pleural surfaces is greatest.[3] Such areas of maximal motion and maximal friction should be the places where friction rubs and pleuritic pain first appear. In our cases the pain was commonly referred anteriorly around the lower rib margins and even to the epigastrium.

We have seen similar precoma patients with abdominal pain which was steady or accentuated by respiratory movements. It is possible that some cases of abdominal pain seen in diabetic acidosis may be referred to the epigastrium from pleural involvement as is often the case in pneumonia. One wonders if dryness of the serous surfaces of the intestines with friction between loops during breathing or from peristalsis could be a contributing cause of the abdominal pain and associated rigidity of the abdominal wall. Rehydration with physiologic saline solution and the administration of insulin in adequate doses tend to abolish these abdominal manifestations quite rapidly.

Experimentally in dogs, Davis[2] found that marked dehydration produces significant and widespread tissue changes. Most of these were vascular in origin and included the endothelial surfaces of the pleura. The latter became a dull hue and were covered with a layer of fibrin. Often there were subpleural ecchymoses. It is possible that similar changes may occur in the dehydrated diabetic patient with ketosis. Toxic factors and anoxemia from circulatory failure may be etiologic agents and may contribute to the genesis of these lesions. Certainly the transudation of the liquid elements of the blood through damaged vascular walls onto the pleural surfaces with the deposition of fibrin seems plausible. Such processes along with the generalized dehydration could conceivably account for the production of acute dry pleurisy in our group of patients.

Summary. 1. Five cases of severe uncontrolled diabetes with acute dry pleurisy have been presented.

2. After insulin administration and adequate hydration were accomplished, the pleuritic pain and friction rubs rapidly disappeared.

3. No conclusive evidence of respiratory disease could be demonstrated, and subsequently the patients remained free of pleuropulmonary disease.

4. Marked dehydration from excessive diuresis and vomiting with resulting dryness of the pleural surfaces is thought to play an important rôle in this manifestation.

5. An analogous situation may exist in the production of abdominal pain and rigidity in diabetic acidosis.

6. Other factors including shock and anoxia may contribute to the tissue changes producing pleurisy.

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ON THE EXPECTORANT ACTION OF VOLATILE OILS*

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There is no more intriguing group of drugs than the class designated as the volatile or essential oils. Most of them have a sensuous appeal, some of them are repugnant, to the senses of smell and taste. Their use in the arts, in commerce and industry, is much more extensive than their use in medicine. Their use undoubtedly extends back beyond the limits of recorded history, for some of them are described in the earliest writings of mankind. Their chemical composition, known in part, still offers a challenge to the efforts of the organic chemist. Their method of formation and their significance in trees and shrubs, in garden plants and in the wild life of the forest and field, offer problems of interest to the plant physiologist. Though many of them have been used in medical practice for thousands of years, we are still largely ignorant of their pharmacologic actions and therapeutic possibilities and continue to wallow in the heritage of dubious, often erroneous, information which has been passed down to us from decades and centuries long since past.

A sub-group of the volatile oils is listed in textbooks of materia medica, pharmacology and therapeutics as having expectorant properties. This group includes a number of volatile oils and their derivatives obtained chiefly from several genera of the coniferæ plus a few medicinal balsams. There are other volatile oils, not ordinarily classified as expectorants, but reported to have expectorant properties, such as oil of eucalyptus.

It was decided to investigate the reputed expectorant action of these drugs by a new method which we have devised and used successfully in this laboratory for some years.[3,10] By this technique, bronchial fluid, or what we prefer to call respiratory tract fluid (R.T.F.), is collected through a cannula in the trachea of an animal lightly anesthetized with urethane, under certain standardized conditions. An increase or decrease in the output of R.T.F. is indicative of an altered production or excretion or both of R.T.F., properties which are ordinarily ascribed to expectorant drugs. Using this technique, expectorants such as ammonium chloride, ammonium carbonate, ipecacuanha, guaiacols, creosotes, camphorated tincture of opium, potassium iodide and certain organic iodides, have been found to augment the output of R.T.F. and other expectorant drugs are being studied.

The present report is of a study involving the application of this technique to the reputed expectorant action of several volatile oils and balsams in some 300 animals, including albino rats, guinea pigs, rabbits, cats and dogs.

The drugs used in this investigation have been described in textbooks for so many years, descriptions which have for the most part been copied from one edition or textbook to another, that we have forgotten—or we have never known—when and why they were introduced into therapeutics in the first place. Modern pharmacologic research has ignored them almost entirely. Under these circumstances, some pay little attention to them and some even consider them as useless relics of the past and little, if any, better than placebos. The commercial slogan that it pays to advertise aptly and amply applies in this case—in the negative, as we shall demon-

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strate in the experiments to be described. In the first group of experiments, a series of volatile oils was given by stomach tube in the doses stated to guinea pigs.

Oleum Terebinthinæ, B.P.; Oleum Terebinthinæ Rectificatum, U.S.P. XII. Crude turpentine is obtained by tapping trees of various species of the genus Pinus. Since it contains resinous and phenolic acids, it is treated with a solution of sodium hydroxide and the rectified oil distilled over. Turpentine was recommended in the Talmud as effective against renal stones.[14] A physician with Columbus on his second voyage to America, described a turpentine as being used by the native Indians.[8] Flückiger and Hanbury[6] described various turpentines which were employed by the ancients and note that the Romans found turpentines being used by the peoples in the Alpine regions of Gaul, by the peasants in the Tirol and by the inhabitants of the Swiss Alps. Turpentine has been used, externally and internally, in the treatment of many human ills. Some 2000 years ago, Pliny[12] is stated to have used turpentine internally against cough. But crude turpentine did not become a very popular remedy, for obvious reasons, until about the 17th century when workers in the regions of Bordeaux and Marseilles prepared oil of turpentine by distillation[12] and some time later the process of rectification was introduced. We have found few references to the internal use of oil of turpentine in textbooks published up to the latter half of the 19th century, but after that time it may be found reported regularly as an expectorant and recently Goodman and Gilman[7] have classified it amongst the more commonly used "stimulant" expectorants.

To the varying fortunes of oil of turpentine as an expectorant, may be added the present, as well as past, confusion in descriptions of its mode of action. Some authors, such as Brown,[5] describe it as a "stimulant" expectorant, tending to stimulate repair of damaged bronchial mucosa and drying up secretions of the respiratory tract. In other textbooks of recent date, the statement may be found that oil of turpentine augments the output of R.T.F. The only reason we can find for these antithetical assertions, is that they must be based upon clinical observations in different types of patients. No satisfactory, quantitative method for measuring the volume output of R.T.F. appeared before the publication of the method used in this laboratory and briefly described above.

Oil of turpentine and the other volatile oils were administered to guinea pigs by stomach tube in the doses stated, 3 hours after the animals had been arranged for collection of R.T.F. and at a time when the output of R.T.F. had assumed a steady, plateau level. The output of R.T.F. was noted at intervals of half an hour for a period of up to at least 4 hours after giving the drug, and the output expressed as ml. of R.T.F. per kilo body weight of animal per 24 hours. Each dose of each drug was given to 7 to 10 guinea pigs, the results were tabulated and averaged, and, to facilitate presentation of the data, any increase or decrease in the rate of output of R.T.F. each hour after giving the drug was calculated as a percentage of the average output during the 2 hours immediately preceding administration of the drug. Results obtained in this manner from the administration of 10, 50 and 100 mg. per kilo body weight of oil of turpentine to guinea pigs, have been collected in Table 1. It was found that oil of turpentine augmented the rate of output of R.T.F. in this species for a period of 2 hours following all doses and for up to at least 4 hours following the 50 mg. dose. Not only was the effect more prolonged following the 50 mg. dose, but it was also more marked, while the increase was not nearly as marked following the larger, 100 mg. dose. These results indicate that oil of turpentine markedly increases the output of R.T.F. in anesthetized guinea pigs and suggest that it may have useful expectorant properties.

Oleum Abietis, B.P. Closely related in origin to oil of turpentine, is Oleum Abietis, B.P., or oil of Siberian Fir, com-

monly called oil of pine. The name oil of pine is somewhat loosely applied to a number of volatile oils, usually prepared by steam distillation, from leaves or needles or cones of various species of Pinus and Abies. *Oleum pini pumilionis*, or oil of dwarf pine needles, is listed in the U.S.P. XII, and is of this class of volatile oils. Oil of pine contains esters of the alcohol, borneol, in addition to various terpenes, while oil of turpentine consists chiefly of pinenes and camphene. Oil of pine is described, chiefly as an inhalant, in textbooks published in the latter half of the last century. Its introduction into therapeutics seems to be related to the belief that some therapeutic virtue lay in having patients with pulmonary tuberculosis live in the region of evergreen forests where they were continuously exposed to inhalation of the coniferous volatile oils.

Oil of pine, in the form of Oleum Abietis, B.P., was given by stomach tube to guinea pigs in doses of 10, 50 and 100 mg. per kilo body weight and the mean percentage effect upon the output of R.T.F. is recorded in Table 1. Oil of pine augmented the output of R.T.F. in a manner somewhat similar to that of oil of turpentine, though on the average the effect was not as striking. As with oil of turpentine, the 10 and 100 mg. doses did not have as marked an expectorant effect as did the 50 mg. dose.

Terebenum, B.P. Terebene is official in the B.P. and while formally official in the U.S.P., it was deleted with the publication of U.S.P. XII. It is prepared by steam-distilling the product resulting from the action of sulphuric acid upon oil of turpentine. It was introduced in the 19th century, apparently with the idea of improving upon the odor and taste of oil of turpentine, which purpose is partially accomplished in terebene. Further justification for its elimination from the U.S.P. is contained in the results obtained in the present work; for, while it did augment the output of R.T.F., the average increases from doses of 10, 50 and 100 mg. per kilo

TABLE 1.—THE EFFECT OF EXPECTORANT VOLATILE OILS AND BALSAMS UPON THE OUTPUT OF R.T.F. IN GUINEA PIGS

Drug	Dose (mg. per kilo)	% increase in output of R.T.F. 1st hr.	2nd hr.	3rd hr.	4th hr.
Oil of turpentine	10	133	89	−44	−47
	50	160	112	93	141
	100	34	61	11	−11
Oil of pine	10	79	−10	−8	21
	50	34	76	101	−18
	100	−16	42	103	26
Terebene	10	88	53	26	−9
	50	59	32	46	5
	100	27	27	47	20
Terpin hydrate	10	0	16	−21	−5
	50	76	38	17	−9
	100	96	26	52	13
Oil of anise	10	194	211	121	105
	50	552	347	275	211
	100	54	19	8	25
Oil of lemon	10	30	49	49	0
	50	240	15	−9	−21
	100	30	53	50	−15
Oil of eucalyptus	10	48	58	52	14
	50	157	172	114	101
	100	88	31	8	4
Friar's balsam	0.05 ml./kilo	−20	−25	0	−29
	2.5 ml./kilo	32	32	−5	−25
Control: 12% alc.	5 ml./kilo	0	−26	8	22

body weight of guinea pig were not as marked as those obtained from the use of oil of turpentine or of oil of pine. These results are shown in Table 1.

Terpini Hydras, U.S.P. XII. Terpin hydrate is a terpene alcohol of known composition listed officially in the U.S.P. XII but not in the B.P. 1932 nor in subsequent Addenda to the B.P., although a related product, terpineol, which is prepared from terpin hydrate, is described in the Sixth Addendum of the B.P. which became official in 1943. Terpin hydrate may be made from oil of turpentine by the action of nitric acid in the presence of alcohol and water under specified conditions. It is a solid with little or no odor or taste, for which reasons it was apparently introduced toward the end of the 19th century, in the textbooks of which time one may find brief reference to it. Terpin hydrate was given to guinea pigs by stomach tube in doses of 10, 50 and 100 mg. per kilo body weight. As shown in Table 1, it did augment the output of R.T.F., but not to the same extent as did oil of turpentine.

These were the 4 terebinthinate volatile oils and derivatives investigated. It is obvious that, of this group, the most effective in augmenting the output of R.T.F. was the original oil of turpentine. None of the more recent, "improved" forms of oil of turpentine was as powerful an expectorant as was the original volatile oil. If our data may be applied to human therapy, they would suggest that oil of turpentine is the most effective of the terebinthinate expectorants and should be given in doses of about 1 ml. by mouth in the form of capsules or perles or in a highly flavored fluid vehicle. To the physician and medical student, the greatest drawback to an intelligent understanding of drug therapy has been, as it is today, the overwhelmingly lost list of compounds of reputed therapeutic value which are presented. With the fund of medical knowledge continuously increasing, it is obvious that continuous pruning is necessary, but the problem has always been what to take out and what to leave in. In the case of drug therapy, factual knowledge is unduly lengthened by having to include lists of drugs in different groups, each list of drugs having more or less the same action. The ideal arrangement would be to select one drug which has been scientifically proven to be the best in the group for general purposes, describe it in detail, and add brief notes upon related drugs which may have some particular virtue in specific circumstances. This process of elimination is not an easy one, but it is, or should be, primarily a function of the researcher in pharmacology and therapeutics. In the present investigation, oil of turpentine was found to be the best terebinthinate expectorant, and should this fact be confirmed, it would be described as typical of the group.

Oleum Anisi, B.P., U.S.P. XII. Our attention was directed next to a few other volatile oils with reputed expectorant properties. The first of these, oil of anise, is prepared by steam-distilling dried, ripe aniseed or the corresponding fruit of the star anise. Anise is one of the most ancient of drugs. The small, annual plant from which it is obtained, originally came from Egypt and the Middle East[12] but later was cultivated in various parts of the world. Charlemagne ordered it cultivated upon his imperial farms in 812.[6] Many of the ancients described it, including Theophrastus, Dioscorides and Pliny. Dioscorides wrote that it sweetened the breath, was diuretic, useful in dropsy, an antidote against various venoms, carminative, effective against leucorrhea, a galactagogue and an aphrodisiac,[12] while Pythagoras recommended that it be held in the left hand as a cure for epilepsy![14] Little wonder that it was widely sought after—and widely taxed. In 1305, Edward I of England set about to raise funds for repairing London Bridge and levied a tax upon a number of items amongst which was anise,[6] while Charles I of England put upon it what was then considered a huge tax but would be looked upon as a mere trifle in this modern Taxing Age.

The star anise is of quite different origin.[6] It comes from a small tree which is native to China and was described in writings of the time of the Sung dynasty. It was introduced into western medicine in the latter 17th century. Exactly when anise was first used as an expectorant, we have been unable to determine, but in a book published in London in 1682,[1] there may be found references to prescriptions containing aniseed and recommended for pleurisy. Oil of anise is present in one of the most effective antitussive drugs which we have today, namely camphorated tincture of opium or paregoric, and it materially contributes to the expectorant properties of that preparation, even though present in only very small amounts.[4]

When given by stomach tube to guinea pigs in doses up to 50 mg. per kilo body weight, oil of anise proved to be the most effective expectorant of all the volatile oils investigated. As shown by the data recorded in Table 1, the rate of output of R.T.F. was increased as much as six fold. Again, a larger dose of 100 mg. per kilo body weight was much less effective than the medium dose of 50 or even the smaller dose of 10 mg. If these data apply to man, they indicate that oil of anise is not being used to the extent that it might be as an expectorant drug.

Oleum Limonis, B.P., U.S.P. XII. The peel of lemons contains numerous cells filled with an essential oil, which is expressed to form medicinal oil of lemon. The lemon tree appears to have been originally native to India and the word, "lemon," is thought to have been derived from "Limu," a Cashmere word.[6] Lemons were unknown to the Greeks and Romans and were introduced into western medicine and commerce by the Arabians, probably about the 11th or 12th century. Oil of lemon has been used for various purposes in medicine, being used now chiefly as a flavoring agent in syrup of lemon. In the form of hot, sweetened lemonade, it is a household remedy for coughs and colds, and this use, the exact origin of which we have been unable to trace, suggested to us the inclusion of oil of lemon in our studies.

The average recommended human dose of oil of lemon is of the order of 2 mg. per kilo body weight. The volatile oil was given to guinea pigs by stomach tube in doses of from 10 to 100 mg. per kilo body weight, and the effect upon the output of R.T.F. is summarized in the data presented in Table 1. As may be seen, oil of lemon augmented the output of R.T.F. and the greatest increase followed the administration of the 50 mg. dose.

Oleum Eucalypti, B.P., U.S.P. XII. Oil of eucalyptus is steam-distilled from the fresh leaves of various species of the eucalyptus tree which is native to Australia. Its chief volatile oil is cineol which, under the name of eucalyptol, is official in the B.P. and U.S.P. XII. Oil of eucalyptus was first used as an adulterant of more expensive, similar oils, such as oil of cajuput which was obtained from a tree native to the region of the Dutch East Indies, which was investigated by the Dutch in the early 18th century and shortly afterwards introduced into European medicine for various purposes, amongst them being as a diaphoretic.[6] One finds little or no reference to oil of eucalyptus in books upon materia medica published before the second half of the 19th century. Wood[13] states that while attention was drawn to the virtues of oil of eucalyptus as early as 1792, it was neglected until after 1860 when eucalyptus trees were cultivated in and around Paris. About that time, the supply of cinchona bark from South America had become seriously limited and large scale production in the Dutch East Indies was not yet under way. As a result, there was intensive research for a substitute for cinchona alkaloids in the treatment and prevention of malaria, and, amongst other drugs, oil of eucalyptus was tried because, "the freedom of Australia from malarial climatic influences," was attributed to the presence there of eucalyptus trees.[13] Eucalyptus trees were planted in several malarial districts and it was reported that portions of the Roman

Campagna were made habitable as a result of cultivation of these trees.[11] All of this naturally stimulated considerable laboratory and clinical research, as a result of which it was soon recognized that, while oil of eucalyptus was inferior to the cinchona alkaloids as an antimalarial drug, it did possess other therapeutic properties amongst which was listed its value in bronchitis, nasal catarrh, and allied respiratory conditions.[13]

The average recommended dose of oil of eucalyptus in man is of the order of 10 mg. per kilo body weight. The volatile oil was given by stomach tube to guinea pigs in doses of 10, 50 and 100 mg. per kilo body weight and the effect of these doses upon the rate of output of R.T.F. has been summarized in Table 1. Oil of eucalyptus was found to be effective in augmenting the output of R.T.F., the maximal expectorant effect being obtained with the 50 mg. dose.

Tinctura Benzoini Composita, B.P., U.S.P. XII. In addition to the volatile oils, a number of balsams, such as benzoin, storax and tolu, have been used as expectorants. The chemistry of the balsams is complex, but as they may be regarded as resinified volatile oils of a particular type, it was decided to include one preparation, compound tincture of benzoin or Friar's Balsam, which contains the 3 balsams mentioned, in the present series of experiments. This tincture was given by stomach tube to guinea pigs in doses of 0.05 and 2.5 ml. per kilo body weight; as shown by the data recorded in Table 1, it had little if any effect upon the volume output of R.T.F. The preparation now known as compound tincture of benzoin has evolved from a number of combinations of balsams obtained from Central America and the Dutch East Indies and introduced in the 17th century.[6,9] Most of these preparations were originally used as vulneraries—some were termed traumatic balsam, wound balsam, and so forth—and were given internally for bronchial catarrh and other conditions. One may find the value of balsams taken internally as expectorants questioned upon clinical grounds in textbooks written toward the middle of the 19th century and our experimental results in animals substantiate the view that balsams, in the form of Friar's Balsam, have little or no expectorant action when taken internally. It is possible that they may have some virtue when administered in the form of a steam inhalation and we plan to investigate this experimentally.

Control Animals. In the above experiments, each drug was dissolved or mixed with 60% alcohol in a concentration such that 1 ml. contained the required dose per kilo body weight. Just before administration, the 1 ml. was mixed with 4 ml. of water, making a total volume of 5 ml. per kilo body weight, which was given by stomach tube. Each dose was given to between 7 and 10 guinea pigs and the results averaged. As a control, a similar group of guinea pigs was given 5 ml. of 12% alcohol per kilo body weight by stomach tube. Changes in the rate of output of R.T.F. were calculated as before and the results have been summarized in Table 1. It may be seen that no marked nor consistent effect upon the rate of output of R.T.F. followed administration of this vehicle.

Species Variation. A study of the pharmacologic actions of a drug ordinarily involves first animal experimentation and then clinical trial. From experiments upon animals, we may ordinarily predict with a fair degree of accuracy what the effect will be in man but we can never be entirely certain until the drug has actually been tried out upon the human subject. In general, it is unwise to predict from experiments upon one species of animals what the effect in man will be. However, if similar or almost similar results are obtained in several species of animals, it is much more likely that the same results will be obtained in man. For this reason, we have selected an exemplary volatile oil, oil of eucalyptus, and studied its effects in various doses and in five species of animals, namely albino rats, guinea pigs, rabbits, cats and dogs. The drug was given as described above and each dose was admin-

istered to from 5 to 10 animals. The results were tabulated, averaged and any effect of the oil of eucalyptus calculated as a percentage effect, as described above. These data have been summarized in Table 2. It may be seen that oil of eucalyptus increased the rate of output of R.T.F. in all 5 species, in some more so than in others, as would be expected. The most effective dose was 50 mg. per kilo body weight. From these data, we may conclude with reasonable certainty that oil of eucalyptus, and hence probably the other active volatile oils studied above, is expectorant in mammals and that the expectorant action probably extends also to man.

tration of most substances in R.T.F. is not very high, ordinarily much lower than in plasma, as previously reported by Boyd *et al.*[2] To overcome these difficulties, R.T.F. was collected and pooled from 5 to 10 animals given a stated dose of the volatile oils noted, and an analysis made of all the R.T.F. put out before, and secondly during the 4 hours immediately after, the giving of the volatile oil. These results have been summarized in Tables 3 and 4. A glance at the data given in these 2 tables will reveal that the several volatile oils in the doses studied had little effect upon the chloride content nor upon the specific gravity of the R.T.F. of the species studied.

TABLE 2.—THE EFFECT OF OIL OF EUCALYPTUS UPON THE OUTPUT OF R.T.F. IN VARIOUS SPECIES

Species	Dose (mg. per kilo)	% increase in output of R.T.F.			
		1st hr.	2nd hr.	3rd hr.	4th hr.
Albino rat	100	62	105	58	−6
Guinea pig	10	48	58	52	14
	50	157	172	114	101
	100	88	31	8	4
Rabbit	10	25	83	10	−14
	50	67	71	21	7
	100	37	14	−7	−8
Cat	10	37	7	14	−11
	50	45	76	86	−3
	100	23	47	33	10
Dog	10	−15	26	51	40
	50	−9	59	58	24
	100	−8	2	−4	−10

The Chloride Content and Specific Gravity of R.T.F. From the data recorded above, we may reasonably conclude that the volatile oils studied act as expectorants and augment the output of R.T.F. In the next series of experiments, we have attempted to ascertain the effect of these volatile oils upon the composition of R.T.F. Since it was not feasible to make a study of all the known physical and chemical properties of R.T.F., we decided to measure only the chloride content and the specific gravity.

The volume of R.T.F per hour, or even per several hours, is ordinarily insufficient to provide an aliquot large enough for the satisfactory estimation of these properties. Added to this is the fact that the concen-

The Mechanism of Expectorant Action. Finally, an attempt was made to find the mechanism of expectorant action of these volatile oils. Oil of eucalyptus was again used as a typical example of the group and it was given to guinea pigs in a dose of 50 mg. per kilo body weight. Four groups of 10 guinea pigs each were used. The abdomen was opened and the afferent gastric branches of the vagus nerve severed in the animals of 2 groups. A laparotomy was also performed upon the animals of the other 2 groups, the intestines manipulated, but the gastric nerves were not severed. Oil of eucalyptus was then given, after the animals had been arranged for collection of R.T.F. for a period of 3

hours, by stomach tube to 1 group of guinea pigs with the afferent gastric nerves cut and to another group with the afferent gastric nerves intact. The remaining group of guinea pigs with the afferent gastric nerves cut and the group with the afferent gastric nerves intact were given a corresponding volume of 12% alcohol and acted as controls. The volume output of R.T.F. after oil of eucalyptus and the vehicle alone was then calculated as a percentage change from the initial output, as before, and the results are summarized in Table 5.

From the data recorded in Table 5, it may be seen that oil of eucalyptus augmented the volume output of R.T.F. to essentially the same extent in guinea pigs with the afferent gastric nerves severed as in those with the nerves intact. There were no significant changes in the rate of output of R.T.F. in either of the control groups. These results indicate that oil of eucalyptus, and probably the other expectorant volatile oils, do not augment the volume output of R.T.F. in guinea pigs, and probably in other mammals, by setting up reflexes to the bronchial glands from the stomach. These expectorant volatile oils do not belong to the class of so-called, "reflex expectorants." The most likely explanation of the expectorant action of these volatile oils is that they directly stimulate the secretory cells of the respiratory tract. There are the less likely pos-

TABLE 3.—THE CHLORIDE CONTENT OF RESPIRATORY TRACT FLUID BEFORE AND AFTER ADMINISTRATION OF VOLATILE OILS

		Chloride content (mg. per 100 ml.)			
Drug	Species	Before	After 10 mg. per kilo	After 50 mg. per kilo	After 100 mg. per kilo
Oil of lemon	Guinea pig	36	36	36	
Oil of pine	Guinea pig	28	28	17	29
Oil of turpentine	Guinea pig	34	..	28	40
Oil of anise	Guinea pig	32	25	32	28
	Rabbit	86	86	71	67
Oil of eucalyptus	Rabbit	73	..	68	77
	Cat	76	65	..	47
	Dog	68	74	102	71

TABLE 4.—THE SPECIFIC GRAVITY OF RESPIRATORY TRACT FLUID BEFORE AND AFTER ADMINISTRATION OF VARIOUS DOSES OF VOLATILE OILS

		Specific gravity of R.T.F.			
Drug	Species	Before	After 10 mg. per kilo	After 50 mg. per kilo	After 100 mg. per kilo
Oil of lemon	Guinea pig	1.083	0.998	0.987	1.009
Oil of pine	Guinea pig	1.015	0.991	0.910	0.990
Oil of turpentine	Guinea pig	1.008	..	0.989	1.005
Oil of anise	Guinea pig	1.018	1.007	1.016	0.982
	Rabbit	0.988	0.989	0.975	0.989
Oil of eucalyptus	Rabbit	0.984	..	0.999	0.997
	Cat	0.993	0.997	..	0.996
	Dog	0.998	0.996	0.997	0.989

TABLE 5.—THE EFFECT OF PREVIOUS SECTION OF THE AFFERENT NERVES FROM THE STOMACH UPON THE OUTPUT OF R.T.F. IN GUINEA PIGS GIVEN 50 MG. PER KILO OF OIL OF EUCALYPTUS

		% increase in output of R.T.F.			
Drug	Afferent nerves	1st hr.	2nd hr.	3rd hr.	4th hr.
Oil of eucalyptus	Cut	221	101	−20	−28
	Intact	168	164	82	70
Control: 12% alc.	Cut	38	−14	32	−34
	Intact	15	3	−29	0

sibilities that the expectorant action is mediated through reflexes initiated elsewhere, such as from the respiratory tract itself, or that the volatile oils stimulate the "expectorant center" in the brain.

Summary. Volatile oils and related compounds have been used as expectorants for many years and as the reason for their introduction into therapeutics has been largely forgotten, their history has been reviewed in some detail. When given by stomach tube to guinea pigs, lightly anesthetized with urethane, the volatile oils of turpentine, pine, anise, lemon and eucalyptus and the related drugs terpin hydrate and terebene, were found to increase the volume output of respiratory tract fluid (R.T.F.). Oil of anise proved to be the most effective expectorant of the group and oil of turpentine the most effective terebinthinate volatile oil. The most effective dose was 50 mg. per kilo body weight. Compound tincture of benzoin, given by stomach tube, had no significant effect upon the volume output of the respiratory tract fluid (R.T.F.). Regarding species variation, oil of eucalyptus, taken as representative of the group, was shown to be expectorant in albino rats, guinea pigs, rabbits, cats and dogs. In a cursory study of the chemical and physical properties of R.T.F., it was found that the expectorant volatile oils had no effect upon the concentration of R.T.F. chloride nor upon its specific gravity. Investigating the mechanism of expectorant action, it was shown that this effect was not influenced by section of the afferent gastric nerves, from which it was concluded that the expectorant volatile oils do not act reflexly from the stomach but probably directly upon the secretory cells of the respiratory tract.

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ALTERATIONS IN CAPILLARY PERMEABILITY IN MENINGEAL IRRITATIONS*

An Aid to Differential Diagnosis

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An increase in capillary permeability is one of the basic tenets of the functional pathology of inflammation. As early as the classic experiments of Cohnheim, dyes were used to demonstrate this phenomenon. The introduction of the fluorescein method[5] has given still more clearly visible evidence of this increased permeability in human beings. A scratch on the skin,[8] a small furuncle, an inflamed vein—these all lead to markedly increased filtration of fluorescein into the interstitial space. When measured with the dermofluorometer,[6] such increased filtration may amount to as high as 400% above normal. It was therefore obvious that similar increases in fluorescein content should be present wherever there is increased fluid transudation into body cavities or tissue spaces in inflammatory conditions.

There are several reports in the pediatric literature about the usefulness of fluorescein in the diagnosis of meningeal changes. They are, however, rather limited in scope because of the patient material, which embraces only a few types of cerebrospinal diseases. Furthermore, because of the early date of these investigations, the technical apparatus were not sufficiently perfected to permit exact estimations of the fluorescein level in the spinal fluid. Kafka,[4] in a continuation of Ehrlich's studies with fluorescein on the flow of the aqueous humor in the eye, studied the questions of the origin and circulation of the spinal fluid. He administered fluorescein orally and took samples of spinal fluid 48 hours later, and noted the fluorescein content of the spinal fluid to be constant throughout the spinal canal. Jervell[3] gave 2 gm. of uranin (sodium fluorescein) by mouth in 74 pediatric cases, and performed lumbar punctures after 3 hours. The fluorescence of the spinal fluid was observed against a black background in daylight. By this rather crude method an increased fluorescein content was noted in 18 cases of "meningitis" not further specified as to origin, in comparison with 56 normal cases. Schoenfeld[9] obtained dubious results with a similar method in cases of general paresis. Esselbruegge[2] injected 0.03 gm. of uranin per kg. intramuscularly in children. Examination of lumbar fluid removed 3 hours later enabled him to make a clear-cut differential diagnosis between meningismus and meningitis. He also stated that in poliomyelitis there was no increase in the fluorescein content of the spinal fluid. Bonar and Bailey[1] administered fluorescein intramuscularly to children and observed the spinal fluid against a dark background after varying intervals. They claimed the method was of great aid in diagnosing epidemic cerebrospinal meningitis.

Our own attention was drawn to this subject when we studied the distribution of intravenously injected fluorescein in various parts of the body, and its relation to capillary permeability. The following methods were used to determine the fluorescein content of the spinal fluid and blood.

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Method. Every patient received intravenously 5 cc. of a 5% solution of fluorescein which also contained 5% sodium bicarbonate.* Exactly 1 hour after the intravenous injection, a spinal tap was performed, and 5 cc. of venous blood was taken. Approximately 1.5 cc. of spinal fluid was needed for the test if the fluorophotometer was used; 0.5 cc. if the visual fluorescence comparator[7] was used. The spinal fluid was then diluted in a proportion of 1 to 10 with distilled water, and the fluorescein content determined. The blood was centrifuged and the plasma diluted 1:100 with distilled water.

A fluorophotometer (Lumetron model, No. 402FF, Photovolt Corp.) was used for the fluorescein determination. The instrument was set at 100 against a standard solution with a fluorescein content of 200 gamma per 1000 cc. The response of the instrument is a straight line in direct proportion to the concentration. The concentration of fluorescein in the spinal fluid can thus be read directly from the calibration of the slide-wire. The plasma concentration is read in the same way.

We are fully aware that the determination of the fluorescein content of the plasma is not quite accurate, since plasma has a fluorescence of its own near sky blue, and this diminishes the fluorescence of fluorescein slightly by shifting it towards blue. This error can be obviated by the use of a blank plasma sample from the same patient, or by employing extraction methods. Such methods were employed only when we needed exact determinations of the fluorescein content of the plasma in connection with other investigations. In the present studies, however, for the purpose of establishing a ratio of plasma:spinal fluid content, we felt that such accuracy was unnecessary, and that simplicity of the method was desirable for usefulness in the clinical laboratory.

When the visual fluorescence comparator is being used, only .5 cc. of the diluted fluids are needed, thus reducing the amount of spinal fluid required. In this instance, a series of increasing dilutions of the standard is kept available for comparison. The concentrations of fluorescein in the spinal fluid and in the blood are thus determined in gamma per 1000 cc., and the absolute values are noted. In order to take into consideration unusually high blood values due to retention in cases with renal damage, the ratio between the blood level and the spinal fluid level was established in each case.

Of 149 patients examined by this method, 45 had no disease involving the cerebrospinal tract. In this "normal" group, the average content of fluorescein in the spinal fluid 1 hour after intravenous injection was 73.2 gamma per 1000 cc. The highest concentration was 400 gamma, and this occurred in the only case exceeding 300 gamma per 1000 cc. The lowest spinal fluid concentration was too low to be determined, and was, therefore, called 0. The average plasma:spinal fluid ratio was 123:1, and the lowest ratio 37:1. Cases in which the spinal fluid content was too low to be determined are excluded from these calculations.

Sixteen cases with cerebral hemorrhage of recent origin showed an average content of fluorescein in the spinal fluid of 73.6 gamma per 1000 cc. The highest concentration in this group was 339 gamma while the lowest was again 0. The average plasma:spinal fluid ratio was 129:1, and the lowest 59:1.

The next group of cases studied comprised 57 patients with either meningovascular or cerebrospinal lues.† This group included cases with high activity, as well as those in the arrested stage. Only those cases were selected in which the diagnosis had previously been established without any doubt. The average fluorescein content in the spinal fluid of these luetic patients was 42.5 gamma per 1000 cc., with the highest being 130 gamma, and the lowest 0 gamma. The average ratio between the plasma and the spinal fluid was 111:1, and the lowest ratio 30:1.

One patient with Froehlich's syndrome was observed in whom the spinal fluid pressure was increased. In this instance, the fluorescein content of the spinal fluid was 180 gamma per 1000 cc., and the plasma:spinal fluid ratio was 43.1.

In 4 cases with meningioma, the average

* Supplied in ampules by C. F. Kirk & Co., New York.

† We are deeply indebted to Dr. Schmitz and Dr. S. Kleiner for permitting us to examine their patients with meningovascular lues at the Manhattan State Hospital.

fluorescein content of the spinal fluid was 170 gamma per 1000 cc.; the highest, 260 gamma; and the lowest, 64 gamma. The plasma:spinal fluid ratio averaged 50:1, while the lowest ratio was 27:1.

The course of meningococcus meningitis was studied in 8 patients. At the height of the disease, the average fluorescein content of the spinal fluid was 439.3 gamma per 1000 cc., with the highest being 1000, and the lowest 155 gamma per 1000 cc. During this acute phase, too, the average plasma:spinal fluid ratio was only 16:1, and the lowest 4:1. In the individual cases of meningococcus meningitis, it was noted that the changes in the fluorescein concentration and ratios mirrored strikingly the clinical course, and significantly did so much earlier than did other laboratory tests. This occurred because the fluorescein tests provide an immediate functional estimate of the inflammatory reaction of the meninges, and are, therefore, not subject to a time lag.

Table 2 illustrates the course of a typical case of meningococcus meningitis from the acute phase to complete recovery. Studies of the 8 cases in this group during the various stages of the recovery phase revealed an average spinal fluid concentration of fluorescein of 171 gamma per 1000 cc., the highest was 455 gamma, and the lowest was 52.6. The average ratio of plasma:spinal fluid in this recovery group was 89.1, with the lowest being 13:1. It must be borne in mind, however, that these cases represented various stages of recovery, some having returned completely to normal.

Pneumococcus meningitis was studied in 1 case. The fluorescein content of the spinal fluid was 526 gamma per 1000 cc., and the plasma:spinal fluid ratio was 8:1.

Our material for the study of tuberculous meningitis was not unequivocal. Three cases were observed of which only 1 was proved by clinical tests as well as autopsy of tuberculous origin. This case had a content of fluorescein in the spinal fluid of 527 gamma per 1000 cc. and a level of 7020 gamma in the plasma. This results in a ratio of 13:1. The second case had a concentration of 1000 gamma fluorescein per 1000 cc. of spinal fluid, while the third with a somewhat doubtful clinical diagnosis of tuberculous meningitis had a content of 83 gamma per 1000 cc. Although it appears highly probable that tuberculous meningitis produces a high permeability, we feel that our data are insufficient to make a definite statement in this direction.

Two cases of severe avitaminosis in alcoholics exhibited a very high content of

TABLE 1.—FLUORESCEIN CONTENT IN SPINAL FLUID

Diagnosis	No. cases	Average (gamma per 1000 cc.)	Highest content	Lowest content	Av. ratio plasma / spinal fluid	Lowest ratio
Normal	45	73.2	400	0	123:1	37:1
"Normal" with cerebral hemorrhage	16	73.6	339	0	129:1	59:1
Meningovascular and cerebrospinal lues	57	42.5	130	0	111:1	30:1
Meningococcus meningitis	8	439.3	1000	155.0	16:1	4:1
Meningococcus meningitis, recovery	8	171.0	455	52.6	89:1	13:1
Pneumococcus meningitis	1	526.0	..	..	8:1	
Tuberculous meningitis	3	537.0	1000	83.0		
Meningioma	4	170.0	260	64.0	50:1	27:1
Severe avitaminosis	2	1330.0	1470	1190.0	3:1	1.5:1
Froehlich's disease	1	180.0	..	..	43:1	

TABLE 2.—PATIENT H. Q. (MENINGOCOCCUS MENINGITIS) SPINAL FLUID

Date	Day of disease	Globulin	Cells	Sugar (mg. %)	Fluorescein (gamma per 1000 cc.)	Ratio of fluorescein plasma / spinal fluid
3/ 4/44	1	++++	6072	35	385.0	12.5/1
3/13/44	9	+	39	78	71.5	48/1
3/16/44	12	−	8	65	56.8	59/1

fluorescein in the spinal fluid, the average being 1330 gamma per 1000 cc., the highest 1470, and the lowest 1190. The average plasma:spinal fluid ratio was 3:1, the lowest 1.5:1.

Discussion. The results detailed in these studies seem to indicate that the fluorescein test, as described, permits a rapid, simple determination of the presence or absence of bacterial meningitis. Many other conditions, such as cerebral hemorrhage, meningismus, cerebrospinal and meningovascular lues, which even on careful examination could easily be mistaken for meningitis, are clearly distinguishable from acute meningitis, since they do not show the high fluorescein content in the spinal fluid found with meningitis.

Among the "normal" cases, there were 4 with meningismus. Meningismus was also present in 2 patients with cerebral hemorrhage. None of these, however, exhibited an increased fluorescein content, thus enabling the observer to make an immediate differential diagnosis between meningismus and meningitis.

Severe avitaminosis leads to a general increase in capillary permeability, as we were able to show in tests on the skin. It is then only natural that this generalized increase should also lead to an increase in the permeability of the vessels of the meninges, thus giving rise to the results obtained in our cases.

Several other conditions, such as acute encephalitis and poliomyelitis, as well as lymphocytic choriomeningitis, require further investigation where such material is available.

Besides being of great value diagnostically, the fluorescein test enables the observer to estimate promptly the success of therapeutic procedures in cases of bacterial meningitis, since there is no time lag such as occurs in the level of protein or cells in the spinal fluid.

Summary. 1. The content of fluorescein in the spinal fluid 1 hour after intravenous injection with 5 cc. of a 5% solution does not exceed 300 gamma per 1000 cc. in a normal individual.

2. With this technique, patients with cerebral hemorrhage have a normal content of fluorescein in the spinal fluid.

3. Meningovascular and cerebrospinal lues also exhibit a normal permeability of the meninges.

4. Cases of meningioma do not show an increase in the fluorescein content in the spinal fluid.

5. Meningococcus meningitis, pneumococcus meningitis, and tuberculous meningitis produce clear-cut increases of the fluorescein content up to 1000 gamma per 1000 cc. The increase parallels closely the clinical course of the patient and seems more indicative of the actual pathology than the usual tests.

6. Severe avitaminosis of the B group also leads to an increase in the capillary permeability of the meninges, with values up to 1330 gamma per 1000 cc.

7. Meningismus does not produce an increased permeability, and thus can immediately be differentiated from meningitis.

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SULFONAMIDE UROLITHIASIS

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SIX years have passed since sulfapyridine-induced uroliths were first described in experimental animals. During these years renal lithiasis has been observed to occur in human subjects as a result of sulfonamide therapy, and methods for the prevention and treatment of renal blockage have been reported, but renal complications still constitute a serious hazard in the use of these clinically valuable drugs. It seemed desirable, therefore, to present a review of the salient features of sulfonamide-induced urolithiasis.

This review divides itself with some overlapping into 4 parts, namely, the pathologic picture, the renal excretion of the sulfonamide drugs, the metabolism or detoxication of these drugs, and finally the factors influencing the prevention and control of urolith formation. These phases of the problem are discussed in order in the following paragraphs, but no attempt has been made to adhere to a strictly chronologic sequence, nor has there been any attempt to include all of the many important papers published in this field.

Pathology. In a short note on poisoning in mice due to "prontosil" (a sulfonamide azo dye of unspecified structure), Oakley[45] described the presence of prontosil crystals in the straight and convoluted tubules and in the urinary bladder. The following year Stewart, Rourke and Allen[63] observed the appearance of sulfanilamide crystals in the urine of patients and suggested the possibility of stone formation in the urinary tract, but it was not until sulfapyridine was introduced into therapeutics that renal complications became significant. Antopol and Robinson[1] announced the formation of acetylsulfapyridine uroliths in experimental animals, and this announcement was followed in the same journal by the report of Gross, Cooper and Lewis[20] which described similar observations.

Antopol and Robinson[2] reported that administration of large experimental doses of sulfapyridine in the rat and monkey lead to urolithiasis. The urolith formation was often unilateral, occurring more frequently on the right side. If the ani-

mal was sacrificed 24 hours after administration of the drug, aggregates of the crystals were often found in the ureter, especially at the level of the bony pelvic brim. After 5 to 10 days of feeding, the concretions were frequently found near the ureterovesical junction or at times in the bladder. In the early part of the feeding period, the ureter was dilated and thinned out. Later, it was indurated and, in some instances, markedly hemorrhagic. The kidney became edematous and enlarged. Bloody urine was found within the dilated portion of the renal pelvis and ureter. In some instances, an amorphous aggregate of crystals and fibrin completely filled the renal pelvis and extended upward into the papillary ducts of the kidney. The bladder was often edematous, especially in the region of the trigone.

These authors reported that, microscopically, the kidney revealed degeneration varying from mild to most severe, especially in the proximal convoluted tubules. With larger doses, the proximal and distal convoluted tubules could not be differentiated from each other. The cytoplasm of the cells forming these tubules was coarsely granular and occasionally vacuolated. Hyaline droplet degeneration was rare. The lumina of the tubules contained globoid eosinophilic bodies. The loops of Henle were markedly distended and contained, in addition to a few globular elements similar to those found in the convoluted tubule, a coagulum which was eosinophilic with hematoxylin and eosin, and blue with azocarmine. In some instances the cells lining the parietal layers of Bowman's capsule were covered by cytoplasm in a cuboid form, in which a nucleus was rarely observed; when one was present, the cell closely resembled a cell of the proximal convoluted tubules. These cells stained red with azocarmine, and transitions between these and the globular red-staining bodies found in Bowman's space were noted. Apparently, these are identical with the globoid bodies described in the tubular lumina. With azocarmine the glomeruli showed marked thickening of the basement membrane of the glomerular tuft. When doses of sulfapyridine insufficient to produce concretions were administered the same degenerative changes were also found, though usually not so pronounced. Following excessive dosage, the glomeruli were markedly distended and contained, in addition to the cell débris, a coagulum similar to that found in the loop of Henle. The corresponding glomerular tufts were compressed, and the capillaries were collapsed and bloodless. The collecting tubules showed only slight to moderate distention. The lumina of the papillary ducts were filled with coagulum, and only a few contained an appreciable number of the globoid bodies described. Other collecting tubules and papillary ducts contained coagulum and fibrin with clefts corresponding to space in which crystals had been present. Many of the tubules contained and were surrounded by a mantle of polymorphonuclear leukocytes, and rarely a foreign body type of giant cell was found between the tubules. Pyelonephritis and purulent periureteritis and peripyelitis were also observed. If the animals were given ample doses over a long period, calcium casts were found in the tubules.

In cases in which calculi were present within the ureter, the ureter and the renal pelvis showed from slight to extremely pronounced edema of the lamina propria, with a moderate diffuse scattering of polymorphonuclear leukocytes. In isolated instances a phlegmon was found. The epithelium for the most part was intact, but an occasional erosion was observed. In those cases in which the renal pelvis was found to be filled with an amorphous collection of fibrin and smaller calculi, the pelvis contained fibrinous material with clear clefts corresponding to spaces in which crystals had been present. In places the transitional epithelium was absent, and in these locations the fibrin rested directly on the lamina propria.

The blood-vessels in these regions showed extreme dilatation.

At times there occurred extremely hemorrhagic ureteritis and pyelitis, far in excess of that which might be expected from the presence of the corresponding calculus. Occasionally, thrombi were encountered in the veins of the kidney. In some instances obliterating lesions were found within lumina of veins which were not in close proximity to any of the inflammatory zones. Intra-ureteral hemorrhage was at times excessive, and in 2 cases the clotted blood caused urinary obstruction. In some instances, after feeding with doses insufficient to produce concretions, the kidneys were edematous and revealed the glomerular and tubular changes described except that no "crystal casts" were found in the tubules. Because of this observation, and because the hemorrhagic reaction may be extreme, the authors concluded that the formation of the urolith may not always be an independent precipitation process, but may, at times, be dependent upon primary degenerative or vascular changes in the kidney.

Upon the introduction of sulfathiazole into the family of sulfonamide drugs, van Dyke and his associates[47,65,66] compared the toxicity of sulfathiazole and sulfapyridine in mice, rats and monkeys. The principal pathologic change noted in all 3 species was renal damage. When incorporated in the diet of the rat, 1% sulfapyridine was toxic. The animals showed concretions in the bladder, ureters or in the kidney pelvis, usually with hydroureter and hydronephrosis. Of the rats receiving 1% sulfathiazole in the diet, 28% showed yellow streaks in the medullæ. These authors concluded that sulfathiazole undergoes less conjugation (that is, acetylation) than sulfapyridine, and this observation was confirmed by Long and associates.[33,34] Although the mouse acetylates little, if any, of the sulfonamide drugs, hematuria and accretions in the bladder were occasionally seen.[47] Thus, it should be noted that renal complications were observed in the absence of acetylation. Gross, Cooper and Scott[21] demonstrated that both sulfamethylthiazole and sulfathiazole cause urolithiasis, and this condition was associated with secondary infection and pyelonephritis which occasionally caused death. A considerable portion of the uroliths was deposited in the renal tubules, sometimes causing urinary block.

Similar pathologic findings were described in due course after the introduction of each new heterocyclic sulfonamide. Crossley and collaborators[11] reported their findings on sulfadiazine. Welch and associates[67] compared sulfadiazine with the more recently introduced sulfamerazine, and Robinson, Siegel and Graessle,[49] extending the observations of Schmidt *et al.*,[51,52] reported studies on sulfapyrazine. These authors observed that rats receiving sulfapyrazine or sulfadiazine revealed essentially the same renal pathology, namely, concretions in the kidney pelvis, ureter and bladder with secondary hydronephrosis, pyonephrosis or pyelonephritis.

The possibility, suggested by Antopol and Robinson, that sulfonamide drugs may produce primary changes in the kidney which predispose to concrement formation has been ignored all too frequently. As the urine passes down the tubules, and water and base are reabsorbed from the glomerular filtrate, the urinary pH drops and the sulfonamide concentration increases. The concentration of the particular sulfonamide excretion product may, thus, exceed its solubility, but precipitation will not necessarily always occur. On the contrary, the supersaturated urine may pass out of the urinary tract in a metastable condition. It is a well-known physical chemical fact, however, that the presence of nuclei, upon which crystals may grow, will cause precipitation from supersaturated solutions. Thus, a primary change in the kidney which furnishes a nidus for crystal growth may determine the presence or absence of concrements within the urinary tract. Further, if the primary changes alter

tubular processes, urolithiasis may occur, whereas it would not occur in a normally functioning kidney. In experimental studies of the newer sulfonamide drugs, Antopol and associates[4,31] emphasized the damage to the tubular epithelium induced by these drugs. Aside from the dilatation of tubules and glomerular spaces and the formation of globoid bodies, they observed severe parenchymatous degeneration of distal convoluted tubules and collecting tubules, with considerable calcification of both of the latter structures. This picture of calcifying nephrosis was seen occasionally after a single injection of sulfathiazole. Hellwig and Reed[25] described a case of a fatal anuria following sulfadiazine therapy and reported that the severe degenerative changes observed in the tubules were similar to those seen in mercuric chloride poisoning. These authors proposed that sulfadiazine is a tubular poison, and that the anuria resulted not because of any failure of renal drainage but because of the tubular damage which permitted a free diffusion of the glomerular filtrate water back into the blood. Wright and Kinsey,[68] in a study of renal complications in a series of human patients who received sulfadiazine, concluded that the drug may produce 2 types of renal complication, one due to mechanical blockage and the other due to a direct damage of the tubular epithelium. Marshall had considered the influence of sulfonamide drugs upon renal function, and long before the observation of urinary concretions, Marshall cautioned against the use of these drugs in the presence of established renal impairment. His fundamental studies on the renal excretion of the sulfonamide drugs are touched upon in the following section.

Renal Excretion. Shortly after sulfanilamide was introduced into experimental therapeutics, Marshall and associates[38] described a colorimetric method for the determination of the sulfonamide drugs in body fluids and tissues. Using this rapid and precise method to great advantage, Marshall established the range of therapeutically effective blood concentrations of sulfanilamide. He soon demonstrated[37] that the true toxicity of a difficultly absorbed drug cannot be determined by the dose of the drug administered orally, but that the toxicity of the drug can be related to its concentration in the circulating blood.[37] His rational approach to chemotherapy, based upon the idea of maintaining therapeutically effective, but toxicologically safe blood levels, was developed by Marshall and, subsequently, by others. It has now become customary, as each new sulfonamide drug is introduced, to define such levels. With the colorimetric method at hand, Marshall and associates[36] studied the renal excretion of sulfanilamide in man and the dog. Taking the simultaneously determined creatinine clearance as a measure of the glomerular filtration rate, Marshall found that the sulfanilamide clearance in the dog was only 20 to 30% of the creatinine clearance. This result indicates that 70 to 80% of the free sulfanilamide is reabsorbed during the passage of the glomerular filtrate along the tubule. In man, the average sulfanilamide clearance was 18% of the inulin clearance, indicating that man, like the dog, reabsorbs large amounts of the drug. The authors found that the sulfanilamide clearance was increased with increasing urine flow, and they stated that the clearance of acetylsulfanilamide is greater than that of free sulfanilamide. In a subsequent communication Marshall and Litchfield[37] reported that excessive blood concentrations of sulfanilamide depressed the creatinine clearance in the dog.

In the course of a study on the excretion of urea, Nicholes and Herrin[44] obtained sulfanilamide clearances which led to the same conclusions as those of Marshall *et al.* And, in essential agreement with the previous work, Loomis and associates[35] found that the sulfanilamide clearance in the rabbit is 30 to 40% of the simultaneously determined creatinine and inulin clearances. These authors re-

ported identical clearances for acetylsulfanilamide, creatinine, and concluded that acetylsulfanilamide is not reabsorbed in the tubules, but is excreted solely by glomerular filtration. These authors also concluded that, in the rabbit, the clearances of both free and acetylsulfanilamide are independent of the urine flow. Similar findings were reported by Shannon.[12]

In a study of sulfapyridine clearance in the dog, Marshall[37] noted that blood levels of 10 to 16 mg. per 100 cc. (maintained by the intravenous administration of solutions of the sodium salt) did not depress the creatinine clearance. And although blood levels of 38 to 45 mg. per 100 cc. produced a temporary depression, the creatinine clearance returned to normal in the course of the experiment. The clearance of free sulfapyridine was not greatly increased by urine flows of 0.2 to 4 cc. per minute. The clearance was found to be 16 to 39% of the simultaneously determined creatinine clearance. Thus, sulfanilamide and free sulfapyridine are excreted in a similar manner. Both resemble urea, but are reabsorbed to a greater extent in the renal tubules. In contrast with these findings, Marshall observed that acetylsulfapyridine similarly administered produced a marked depression in the creatinine clearance. The clearance did not return to normal within 24 hours, and concretions were found in the renal tubules at autopsy.

In an extensive survey, Shannon and associates[12] reported their studies on the distribution and renal excretion of a large series of sulfonamide drugs in the dog. In their experiments, fasting animals were given water to insure an adequate diuresis and creatinine plus the sulfonamide under investigation were administered subcutaneously in doses sufficient to produce satisfactory blood levels of both substances. Blood and urine samples were then collected and analyzed. The glomerular filtration rate was taken as equal to the mg. of creatinine excreted per minute divided by the plasma creatinine concentration in mg. per cc. The sulfonamide data were manipulated as follows: of the plasma concentration, the filterable sulfonamide concentration in mg. per cc. was determined. This concentration multiplied by the glomerular filtration rate gives the number of mg. of sulfonamide filtered each minute. The product minus the number of mg. of sulfonamide actually found in the urine equals the amount reabsorbed. The ratio of the amount excreted to the amount filtered yields a figure called the *excretion ratio* which is characteristic of the excretion of the compound by the average nephron under the conditions of the experiment. An excretion ratio of 1 indicates that the renal tubules do not participate in the excretion of the drug. A ratio that falls progressively below 1 indicates a progressively increased reabsorption in the renal tubules. A ratio above 1 indicates tubular secretion, the extent of which is reflected in the magnitude of the excretion ratio.

The data in Table 1, taken from Shannon,[12] indicates that sulfapyridine, sulfadiazine and sulfamerazine are reabsorbed to a considerable extent, whereas sulfathiazole appear to be excreted by a process which is almost exclusively that of glomerular filtration. The reabsorption is considerable in the case of sulfamerazine and, as in the case of p-aminobenzoic acid, is of an order of magnitude which suggests that reabsorption proceeds as an active tubular process.

In a more recent study Beyer *et al.*[7] reported the average renal clearance values, corrected for plasma binding, of 4 unconjugated sulfonamides to be 7.1 for sulfamethazine, 9.3 for sulfamerazine, 15.8 for sulfadiazine and 35.4 for sulfathiazole. These workers noted that the administration of sodium bicarbonate increased the urinary pH but also increased the clearance of all of the compounds by interfering with their reabsorption. This effect was most striking in the case of sulfathiazole where reabsorption of the compound in the renal tubule was almost completely inhibited. Thus, the use of sodium bicarbonate to increase the solubility of sulfon-

amide drugs is not without objectionable features, for as the solubility of the drug is increased, the natural tubular reabsorption of the compound is decreased and this latter effect tends to counteract the former. The authors noted that the production of a water diuresis also increased the clearance of each compound but not to so great an extent as followed the administration of sodium bicarbonate. When the plasma level of the sulfonamide was raised through the ordinary therapeutic range, the authors observed a progressive increase in the tubular reabsorption of the compounds with the result that the clearance values did not tend to change with the plasma levels. This observation serves to emphasize the importance of maintaining an optimum blood concentration of the drug.

Table 1.—Excretion Ratio of the Sulfonamides

Compound	pKa	Sulfonamide / Creatinine clearance ratio	% filterable plasma sulfonamide	Excretion ratio
Sulfanilamide	10.43	0.27	90	0.30
p-Aminobenzoic acid	4.68	0.18	93	0.19
Sulfapyridine	8.44	0.38	69	0.55
Sulfathiazole	7.12	0.40	40	1.00
Sulfamethylthiazole	7.80	0.25	23	1.09
Sulfadiazine	6.48	0.27	83	0.33
Sulfamerazine	7.06	0.13	61	0.21
N_1-sulfanilamide—acetic acid ($NH_2C_6H_4SO_2NHCH_2COOH$)	3.52	1.35	92	1.47

The clearance studies considered in the foregoing paragraphs are fundamental to an understanding of sulfonamide urolith formation. It is evident, however, that the investigators, for good and sufficient reasons, have limited their clearance studies. Usually, conditions were imposed which permitted the study of the free drug uncomplicated by other urinary excretion products. Marshall has considered the clearance of both the free and acetylated forms, but it is not yet possible to consider the complete clearance picture. As shown in the following section, the metabolism of the sulfonamide drugs is quite complex and products other than the free and acetylated drug are eliminated in the urine. Since these additional products are concerned in the etiology of urolithiasis, it will be desirable to incorporate them into the clearance picture when analytical methods become available.

Metabolism. Following oral administration, the sulfonamide drugs pass, in part, into the portal circulation and reach the liver. Here, they undergo a series of metabolic reactions which determine to a large extent whether or not the products of these reactions will form uroliths. The products of these reactions, which appear in the urine, may be divided into a *relatively water-insoluble* group and a *water-soluble* group.[55] The former includes those products which may precipitate in the urinary tract, whereas the water-soluble group includes those products which do not form uroliths. Information concerning these detoxication products is, therefore, of fundamental importance in the etiology of sulfonamide urolithiasis.

Within the relatively water-insoluble group are the parent drug, the N_4-acetyl derivatives, hydroxyl derivatives and possibly other as yet uncharacterized urinary excretion products of the sulfonamide drugs. By direct isolation and identification of the urinary product, Marshall[38,39] and others[5] demonstrated that the sulfonamide drugs are, in part, excreted unchanged. Antopol and associates[3] were able to produce massive precipitation of the unchanged sulfonamide within the urinary tract by means of intravenous administration of the sodium salts of a number of different sulfonamides. More recently Welch[67] and others[22] have observed precipitation of free sulfadiazine

and sulfamerazine in the kidneys of dogs. Thus, the unchanged drug may form the urinary concrement. Marshall[38,39] and others[5,48] demonstrated that the sulfonamides, like p-aminobenzoic acid,[10] are acetylated in various animal species, and Harris and Klein[23] described the acetylation of sulfanilamide by liver slices *in vitro*. It has been amply demonstrated that the acetylated derivative of sulfapyridine is more insoluble than the parent drug, and the first sulfonamide uroliths described[1] were composed almost entirely of N_4-acetylsulfapyridine. Many years ago, Schmiedeberg[53] reported that aniline is oxidized in the animal organism to yield p-aminophenol. It was reasonable to assume, therefore, that the sulfonamides, which are homologues of aniline, would undergo a similar oxidation. This reasoning led to the search for and the isolation of a hydroxy-sulfapyridine from dog urine,[56,57] and led more recently to the isolation from rabbit and monkey urine of a hydroxy-sulfaquinoxaline which was shown to be 2-sulfanilamido-3-hydroxy-quinoxaline.[59] Uroliths composed of the almost pure hydroxysulfaquinoxaline were observed in the rat and monkey. Thus, this group of *relatively water-insoluble* products consists of the parent drug, N_4-acetyl derivative, hydroxyl derivatives* and possibly others as yet uncharacterized excretion products, and any one or mixtures of these may form kidney stones.

The water-soluble group of urinary excretion products has not been investigated as intensively as the previous group, but in view of the direct and inferential evidence accumulated there can be no reasonable doubt of the existence of this important class of excretion products. Schmiedeberg[53] demonstrated that the p-aminophenol resulting from the *in vivo* oxidation of aniline was conjugated as an ethereal sulfate, and Külz[30] showed that the p-aminophenol might also be conjugated as a glucuronide. In analogy with this early work, it was logical, following the isolation of the hydroxysulfapyridine, to seek an ethereal sulfate or a glucuronide, and a highly water-soluble hydroxysulfapyridine glucuronide was isolated.[56,57] Shortly thereafter, Thorpe and Williams[64] announced the isolation of glucuronides of a hydroxysulfathiazole and a hydroxysulfanilamide. These direct isolation experiments have been substantiated by inferential studies. Assuming, logically, that the urinary output of a hydroxysulfapyridine glucuronide would be reflected in an increased output of urinary glucuronic acid, it was shown that human subjects receiving sulfapyridine excreted large amounts of the drug in this soluble form.[60] James'[26] findings indicate that sulfanilamide is oxidized *in vivo* to a phenol and is then conjugated as an etheral sulfate. Shelswell and Williams[62] found that 6 to 12% of the sulfanilamide fed to rabbits gives rise to a phenol which is conjugated and excreted as an ethereal sulfate. More recently,[55] a simple distribution method was introduced for the study of these excretion products and by means of this method it was shown that, of the "free" sulfonamide (*i. e.*, not including acetyl derivatives) found in rat urine, 40% of the sulfapyridine; 10 to 20% of the sulfanilamide, sulfathiazole and sulfamerazine; and 4 to 6% of the sulfadiazine and sulfapyrazine appears in the form of water-soluble metabolites. The occurrence of similar soluble excretion products were detected in the urine of the rat, rabbit, dog, and man following the oral administration of sulfaquinoxaline.[59] Thus, there can be no reasonable doubt of the existence of this class of water-soluble excretion products

Since different animal species metabolize a given drug differently, it is important to know how the sulfonamides are

* The bacteriostatic properties of these products are unknown; yet, it would seem important to know if they possess full antibacterial activity. The products are aromatic amines, and as such they diazotize and couple directly. Consequently, they are measured in mixtures together with the parent drug. If they do not possess bacteriostatic properties, analyses of mixtures will give erroneous information.

detoxified in the human subject. Quite recently, Gilligan,[17] studying the urinary output of ethereal sulfate and glucuronic acid following sulfanilamide, sulfapyridine, sulfathiazole, sulfadiazine, sulfapyrazine, sulfamerazine, and sulfamethazine, reported that in no case was the ethereal sulfate output augmented by the drug. The urinary glucuronic acid output was not increased following sulfanilamide, sulfadiazine or sulfapyrazine, but the ouput was greatly increased following sulfapyridine, sulfamerazine, and sulfamethazine. Stoichiometric calculations indicated that 40% of the total sulfapyridine and 68% of the sulfamethazine appeared in the urine as a water-soluble glucuronide.

When as much as 40 to 68% of a sulfonamide drug appears in the urine as as metabolite rather than as the parent drug, one must consider the implications. Quite clearly, distribution studies, and renal clearance studies which are based upon the original drug are not adaptable to metabolites of that drug. Further, the bacteriologic properties of these metabolites should be known because they are measured in current analytical procedure together with the unchanged drug. If the metabolites are devoid of activity, analytical results may be in error by as much as 40 to 68%, and such a discrepancy may have serious consequences. On the other hand, if the water-soluble metabolites retain the antibacterial activity of the parent drug—and unpublished work suggests that the hydroxysulfapyridine glucuronide retains antipneumococcal activity in the mouse—then, they may be particularly effective in the treatment of urinary tract infections because of the absence of urolith formation. Further, the toxicologic properties of these metabolites may be widely different from those of the unchanged drug. Indeed, certain side-reactions, such as methemoglobin formation, may be more readily attributable to metabolic products than to the drug originally administered. Certainly, the existence of a water-soluble group and a relatively water-insoluble group of urinary excretion products, appears to be clearly implicated in the etiology of sulfonamide urolithiasis.

As noted above, products of the water-soluble group do not form uroliths. Hence, each portion of the drug so excreted, reduces the amount of the drug available for urolith formation, and it was suggested[41] that any means which may be employed to increase the percentage of the drug excreted in a water-soluble form would decrease the probability of urolithiasis. Conversely, any interruption of the chain of metabolic reactions which yield these products increases the amount of the drug to be excreted in some other form. That interruption of this chain of reactions may lead to an increased incidence of urolithiasis was demonstrated by chloroform and phosphorus liver damage in the rat. Graded damage of the liver reduced the urinary output of hydroxysulfapyridine glucuronide, and the incidence of uroliths was increased from 10 to 25 to 60%. More recently, an increased incidence of renal complications was observed in the course of sulfonamide therapy among patients suffering of liver disease.[46]

Factors Influencing the Prevention and Control of Urolith Formation. After Antopol and Robinson[1] announced the formation of acetylsulfapyridine uroliths, there was renewed interest in Marshall's earlier studies[36] of the renal clearance of the sulfonamide drugs. The rapid clearance of acetylated sulfapyridine plus the tubular reabsorption of the unchanged drug suggested a mechanism whereby almost pure acetylated sulfapyridine would be precipitated within the urinary tract. If only the acetylation process could be brought under control, the kidney stone problem would be solved.

A solution along these lines was sought by a number of investigators. It was known from the work of Ellinger and Hensel[10] that p-aminobenzoic acid is acetylated in the rabbit, and Sherwin *et al.*[8,24,43] had considered dietary meas-

ures which increased the output of acetylated p-aminobenzoic acid in experimental animals. Extending this knowledge, Harris and Klein[28] demonstrated the *in vitro* acetylation of sulfanilamide by liver slices, and they reported that the factor limiting the rate of acetylation was the speed of acetate production in the tissue slices. James[27] fed sodium acetate, together with sulfanilamide and with sulfapyridine, and noted a decrease in the toxicity of the drugs, but this decreased toxicity cannot be attributed to a decreased acetylation because the mouse, the experimental animal used by James, does not ordinarily acetylate the sulfonamides. Martin and his associates[41] fed a large series of "detoxifying chemicals" to mice receiving doses of sulfanilamide, sulfapyridine and sulfathiazole, and noted that many substances reduce the toxicity of the drugs. Martin[42] extended this work and found that administration of glucuronic acid completely inhibited acetylation of sulfonamides in the rat. Martin suggested that administration of glucuronic acid, in accordance with the Mass Law of Reaction, drove the *in vivo* detoxication reactions toward the formation of the water-soluble hydroxysulfapyridine glucuronide,[56,57] and thus decreased the tendency toward urolith formation by reducing the amount of the drug excreted as the insoluble acetysulfapyridine. It should be noted, however, that the hydroxysulfapyridine glucuronide is not formed unless the sulfapyridine is first oxidized to the hydroxyl derivative.[61] Oral administration of glucuronic acid will not, presumably, accelerate the initial oxidative reaction. After the drug is oxidized, the hydroxysulfapyridine is converted to the glucuronide by way of two 3-carbon moieties[32] rather than by way of one 6-carbon glucuronic acid molecule. These observations cast some doubt upon Martin's hypothesis, but there can be no doubt of the possibility, if not indeed the probability of a decreased toxicity brought about by dietary means. The influence of the diet upon the toxicity of drugs, as well as the converse, namely, the influence of drugs upon the nutritional state of the host are important topics of current interest, but these topics are much too broad to warrant consideration here. It will suffice to suggest that the dietary may prove to be a useful road to the control and prevention of sulfonamide-induced kidney stones, but this interesting avenue of approach awaits further exploration.

In much of the early work there was a tendency to compare new drugs with old. Investigators sought new drugs which would undergo very little acetylation *in vivo*, the assumption being that a lessened degree of acetylation would parallel a lessened incidence of renal complications. For example, Long and his associates[33,34] extending the work of van Dyke,[65,66] reported that less of the acetylated drug was found in the urine of rats, monkeys and human subjects after the administration of sulfathiazole than after administration of sulfapyridine. Similarly, Welch and co-workers[67] found a greater percentage of acetylsulfamerazine than of acetylsulfadiazine in the urine following administration of the 2 parent drugs. Directing his attention to blood concentrations, Welch concluded that the degree of acetylation does fluctuate considerably, but he also concluded that the blood concentrations of acetylated drug remain low because of rapid elimination by the kidneys rather than because of difficulty in the acetylation of the drugs. This entire point of view has given way to a detailed consideration of the solubility properties of newer and more suitable sulfonamide drugs.

The newer drugs, sulfadiazine,[11,50] sulfamerazine,[67] sulfapyrazine,[40] etc., possess interesting solubility characteristics. Unlike sulfapyridine and sulfathiazole, the newer sulfonamides yield acetylated derivatives which are slightly more soluble than the parent drugs. This fact has been excessively stressed. In stressing this fact it is implied that, because of the increased solubility of the acetylated prod-

uct, these drugs will produce a lowered incidence of urolithiasis. The truth is that these new drugs, when properly administered, do yield a lowered incidence of stones, but this cannot be attributed simply to the increased solubility of the acetylated derivative as compared to the parent drug, for, after all, the solubility differences are relatively small. In no case is the solubility of the acetylated product much greater than twice that of the parent drug at the pH of body fluids. If one considers that the aceylated derivatives are not extensively reabsorbed, but tend, rather, to be cleared rapidly in the kidney, this solubility difference grows somewhat in significance, but when one considers that the parent drug, a hydroxyl derivative, the acetylated sulfonamide, or mixtures of these products may be precipitated in the urinary tract, it is clear that the newer sulfonamide drugs do not owe their successful application solely to a slight difference in the solubility of the free and acetylated products.

When acetylsulfapyridine uroliths were first reported, Antopol and Robinson[1] suggested that the administration of large volumes of water caused solution or removal of the stones. This concept has been universally accepted and all workers, both experimental and clinical, require a controlled daily fluid intake. It was but one step from increasing the urine volume to increasing its pH in order to increase the amount of sulfonamide in solution, and thus to reduce the amount of the sulfonamide precipitated in the urinary tract. The full utilization of the pH variable, however, awaited the synthesis of suitable sulfonamide drugs.

Gelmo,[16] who first synthesized sulfanilamide, recognized that this compound was both a weak base and a weak acid, and as such would form relatively water-soluble salts with acids and bases. While these properties were generally known and hydrochlorides and sodium salts had been prepared,[15,58] Marshall's preparation of the sodium salt of sulfapyridine for therapeutic administration[39] appears to have focused clinical attention upon the salt-forming properties of the sulfonamide drugs. Since it is possible to form water-soluble sodium salts for parenteral administration, it is also possible to dissolve uroliths by administering alkali. Reasoning thus, Kawaichi and Barnes[29] recommended alkali therapy in the treatment of renal lithiasis resulting from sulfonamide drugs, and Climenko and Barlow[9] reported that renal impairment induced in the monkey by sulfathiazole could be prevented by the administration of sodium bicarbonate, and Schwartz *et al.*[54] considered the effect of alkali on the crystalluria produced by sulfathiazole and sulfadiazine.

It was not, however, until the newer sulfonamide drugs were synthesized that alkali therapy was placed upon a quantitative basis. Bell and Roblin,[6] in a fine theoretical paper, reported the anionic dissociation constants of a large series of sulfonamide drugs. These constants qualitatively express the relative solubilities of the drugs at a fixed temperature and at a pH fixed within the range of anionic dissociation. They also indicate to what degree the solubility of a given sulfonamide may be expected to increase with increasing pH increments. Fox[13] measured the solubility of sulfonamide drugs in a series of buffers, and in conjunction with others,[14,28] discussed the prevention of renal obstruction by alkali therapy. Similar studies were reported by Gilligan and others.[18,19] In general, these studies are based upon simple pH-solubility relationships. The salient features of these relationships are illustrated in Figure 1.

Curves 1 and 2 may be taken to indicate the type of solubility data obtained with 2 different classes of sulfonamide drugs. The first class includes sulfanilamide, sulfapyridine and sulfathiazole. The second includes the newer drugs such as sulfadiazine, sulfamerazine, sulfapyrazine, etc. As shown in Figure 1, the solubility of the drugs in both classes increases with increasing pH, but the solubility characteristics of the 2 kinds of drugs differ in one very important aspect. The solubility of

drugs in the first group is not greatly enhanced with increasing pH unless the alkalinity exceeds the physiologic range. These drugs undergo some slight dissociation as acids at the pH of body fluids and alkali therapy may help to prevent precipitation within the urinary tract, more particularly in the case of sulfathiazole, but the influence of alkali cannot be great. On the other hand, the solubility of the drugs in the second class is decidedly and favorably influenced by alkali therapy. These drugs undergo a marked increase in solubility with pH increments falling within the physiologic range and precipitation of these drugs within the urinary system can, within limits, be prevented by increasing the alkalinity of the urine.

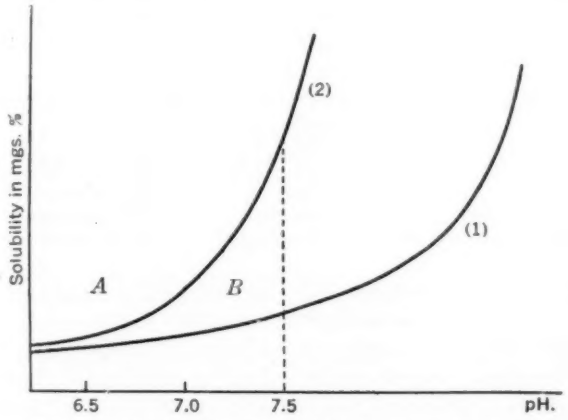

FIG. 1.—pH-solubility relationship of sulfonamide drugs.

An example will serve to illustrate the response of both classes of drugs to alkali therapy. Following medication, the drug is absorbed and a blood concentration is built up. On passing through the glomeruli the unbound drug passes into the protein-free filtrate at a pH of about 7.4. Following the reabsorption of water and base in the tubules, the urinary concentration of the drug may climb as high as 2 to 300 mg. % and the pH may fall as low as 5.5. If the solubility of the drug is exceeded at any point along the way, precipitation may occur. Referring to Figure 1, it is clear that if the urine possesses a concentration and pH indicated by *A*, precipitation may be anticipated. If, however, alkali is administered together with the sulfonamide and the pH of the urine is increased to *B*, the sulfonamide concentration remaining as before, precipitation may or may not occur. If the drug is a member of the second class, point *B* will fall below the solubility Curve 2. In other words, the urine is not saturated, and precipitation will not occur. But if the drug in question is a member of the first class, point *B* will remain above the solubility Curve 1. The urine is supersaturated, and in the absence of metastability precipitation will occur.

In the foregoing example only the unchanged sulfonamide drug was considered. There is no need to complicate the example by individual consideration of each member of the relatively water-insoluble group of excretion products. In each case the excretion products give rise to similar solubility curves and the same argument holds. This is certainly true of the acetylated sulfonamides, and while no solubility data are available for the hydroxysulfonamides it can be predicted that these substances will yield similar if not more pH-sensitive solubility curves. It may be concluded that with the newer and more acidic sulfonamide drugs, adju-

vant alkali therapy is a powerful aid in the control and prevention of stone formation.

There has been an evident tendency to reason by analogy and to generalize experimental findings in the sulfonamide field. This attitude is usually justified, and in many instances is quite necessary; but analogy and generalization tend to obscure important differences between the individual sulfonamide drugs. Some differences between sulfapyridine, sulfathiazole and sulfadiazine are apparent in the comparative data included in Table 2. The use of adjuvant alkali therapy in the control and prevention of urolithiasis has been considered in preceding paragraphs. It is immediately evident from the data in Table 2, that sulfapyridine will not respond to alkali; sulfathiazole will respond but to a slight degree and sulfadiazine will respond strikingly to alkali therapy. The renal clearance of various sulfonamide drugs was considered in the preceding section. It will suffice here to point out that the different drugs differ in their mode of excretion. The extensive reabsorption of free sulfapyridine and the rapid clearance of acetylsulfapyridine accounts nicely for the fact that the uroliths are composed almost entirely of acetylsulfapyridine. The lack of tubular reabsorption of sulfathiazole suggests that this drug will give rise to concrements consisting of a mixture of unchanged and acetylated sulfathiazole. The extensive reabsorption of sulfadiazine reduces the urinary output of the unchanged drug and, depending on the relative concentrations of free and acetylated sulfadiazine, precipitation in acidic urine may produce concrements of one or the other or mixtures of both excretion products. The metabolism of the sulfonamide drugs was considered in a preceding section where it was pointed out that water-soluble excretion products do not form uroliths, and that each portion of the drug so excreted reduces the amount of the drug available for urolith formation. It appears from the data recorded in Table 2 that the urinary output of such water-soluble products will be an important consideration when sulfapyridine is administered. It may be significant with sulfathiazole but will not be significant when sulfadiazine is administered. These differences, then, are of fundamental importance in the etiology of sulfonamide urolithiasis.

Conclusion. With the newer and more acidic sulfonamide drugs, it has been posf sible to reduce greatly the incidence o urolithiasis by control of the daily fluid output and by adjuvant alkali therapy; but other factors and measures are important. Because an established renal or hepatic dysfunction may predispose to sulfonamide urolith formation, these conditions should be considered in conjunction with the administration of the sulfonamide drugs. Perhaps most important in the control and prevention of sulfonamide urolithiasis is strict attention to the blood concentration of the drug. Therapeutically effective, but toxicologically safe, blood concentrations should be maintained throughout the course of therapy. It remains for the clinician to maintain these blood levels by appropriate dosage and adequate diuresis, for as Marshall states, the dose and "the efficiency of the kidneys in excreting the drug . . . are the most important of these factors for the human subject."

TABLE 2.—SOME DIFFERENCES IN THE PROPERTIES OF SULFONAMIDES

Drug	pK[8]	Solubility increase produced by increasing pH from 6 to 7.4	Behavior of unchanged drug in renal tubule[12]	% of urinary products appearing in water-soluble form[48]
Sulfapyridine	8.44	*Nil*	Reabsorbed	40
Sulfathiazole	7.12	Slight	Not reabsorbed	15
Sulfadiazine	6.48	Marked	Reabsorbed	5

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RADIOLOGY

UNDER THE CHARGE OF

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ROENTGENOLOGIC CHANGES OBSERVED IN TROPICAL DISEASES

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MANY of those who were giving serious consideration to the prospect of armed conflict between the United States and Japan anticipated an increase in the incidence of tropical diseases in this country. As was foreseen, large numbers of our military and civil personnel were obliged to enter and remain in areas where these diseases are endemic. Many of them became infected and of these some were returned as invalids, carriers of the diseases, or both. Whether or not this is having a significant effect on the general health of our population is probably not definitely known even in official public health circles; but it is more than probable that the civilian physician will be seeing, now and then at least, patients whose ailments are directly or indirectly related to tropical infection of one kind or another. Defining tropical diseases as those which occur or tend to be more prevalent in tropical and subtropical zones than elsewhere, Garland[6] made a review of the principal ones likely to turn up in this country at the present time or in the near future. "Before a disease can be diagnosed, it must be thought of;" therefore he stressed the pathologic changes which might be encountered at roentgenologic examinations, but he also made brief comments on their clinical manifestations and on some of their epidemiologic features. The discussion has interest and merit not so much because the facts it relates are new, but because they are related from a new point of view, and will serve to keep the reader mindful of diagnostic possibilities to which his habits of thinking may not be accustomed.

Malaria, Garland noted, is probably the most important world-wide disease of modern times and was undoubtedly the major medical problem of the second world war. Protean in its clinical manifestations, it can mimic numerous diseases of different organs of the body, and so patients with malaria may be referred for roentgenologic examination of almost any region. Most of the victims know that they have had the disease and are able to recognize many of its diverse manifestations themselves. In others, however, the disease may have gone long unrecognized until they have: (1) atypical fever resembling that of tuberculosis or typhoid fever; (2) delirium or convulsions; (3) abdominal pain, simulating appendicitis, cholecystitis or intestinal obstruction; (4) diarrhea; (5) pulmonary symptoms, such as those of bronchitis and pleuritis; or (6) nephritis, sometimes with hematuria. Garland repeated the well-known clinical aphorism to the effect that malaria should be held suspect in any person returned from the tropics who becomes ill. The diagnosis is made, of course, with the demonstration of the specific Plasmodium in the blood smear.

Malaria is one of the diseases in which enlargement of the spleen frequently occurs. Garland reported on 3 instances in which the correct diagnosis of malaria was first suggested when a notation of splenomegaly was made at roentgenologic examination of the urinary tract. The

patients were aware neither of the infection nor of the enlarged spleen.

When is the spleen to be considered enlarged? There is a normal variation in size in different persons and from time to time in the same person. Estimates of enlargement must be made with these points in mind. Garland expressed the belief that a roentgenologic estimate will inevitably be more accurate than a clinical one, but he also pointed out that a uniform technique of projecting the organ on roentgenograms must be developed and adhered to. He established the normal size of the spleen in healthy young men by examining a series of them in the supine position. The distance between the target of the Roentgen ray tube and the Roentgen ray film was 36 inches (91 cm.). The length of the spleen was measured on a line drawn through its shadow somewhat diagonally between the upper and the lower poles. The breadth was measured on a line drawn at right angles to the line between the poles through the widest portion of the shadow. In normal persons the length varied between 8 and 16 cm.; the breadth between 4 and 9 cm. The average length of the shadow of the spleen was 12 cm., the average breadth was 6 cm. When the shadow of the spleen was obscure or indefinite in outline, which happened in about 15% of the subjects, it was apparently possible to obtain satisfactory measurements after the colon or the stomach had been distended with air or other gas to enhance the contrast between the spleen and these neighboring organs. The fact of splenic enlargement was determined with reasonable certainty when the shadow was more than 17 cm. in length. If the length was within normal limits, splenic enlargement was determined when the breadth was more than 9 cm. On rare occasions the spleen had the shape of an inverted U, and this made the problem of measurement more complex.

Serial roentgenologic examinations of the spleen also were made on a group of men 18 to 45 years of age who had malaria of less than 2 years duration. The size of the spleen was determined readily in 85% of cases. Garland advocated measurements of the kind described whenever exact determinations are considered to be necessary or desirable. Norms like those Garland established for young men should be established also for young women and for other age groups of both sexes, because the question of splenic enlargement is frequently raised in standard medical practice and such data might well prove to be useful.

Hepatomegaly is also observed in malaria, but aside from this and splenomegaly, cerebral calcification is the only pathologic malarial change of special roentgenologic interest. Apparently, cerebral calcification occurs only in malaria of great severity and of long standing, and particularly in that variety of the disease caused by *Plasmodium falciparum*. Garland made roentgenologic examinations of the skull in a small number of cases of cerebral malaria and in a larger number of cases of chronic malaria, none of them of more than 3 years' duration, but failed to find evidence of calcification in any instance. He noted that numerous other calcifying parasitic, inflammatory and degenerative lesions of the intracranial structures would have to be considered in the diagnosis of such calcifications.

Other tropical diseases associated with hepatosplenic enlargement noted by Garland are leptospirosis, African trypanosomiasis and leishmaniasis. Hepatomegaly without enlargement of the spleen is seen with amebiasis and the hepatic distomiasis caused by *Clonorchis sinensis*.

Tropical diseases manifested chiefly by intestinal symptoms and signs are bacillary and amebic dysentery, hookworm disease, strongyloidiasis, ascariasis, less frequently filariasis, schistosomiasis and the intestinal form of distomiasis, especially that caused by *Fasciolopsis buski*.

Bacillary dysentery, caused by one of several species of Shigella, is encountered the world over, sporadically and in epi-

demics. Since cases of acute bacillary dysentery are seldom referred for roentgenologic examination of the intestinal tract, the manner in which the pathologic changes of this phase of bacillary dysentery might be reflected in roentgenologic findings is not known. Garland noted that in the chronic stage roentgenologic changes resembling those of idiopathic chronic ulcerative colitis have been reported. A chronic form of bacillary dysentery may occur in the tropics. It must be very rare in temperate latitudes. Numerous attempts have been made to show an etiologic relationship of the Shigella organisms to undoubted cases of the disease commonly identified as chronic ulcerative colitis. To my knowledge none of them have been successful. The evidence that has been mustered has been neither consistent nor convincing. In fact, there is reason to doubt the very existence of a chronic form of bacillary colitis which might have a confusing similarity to chronic ulcerative colitis.

Endamœba histolytica causes chronic ulcers in the colon, and amebiasis is encountered with moderate frequency even in temperate latitudes. It is, of course, more prevalent in the tropics than in the temperate zone. Many of our military personnel became infested while in tropical zones, and some of them returned to this country as carriers or with amebic dysentery. The same pathogen causes hepatic and pulmonary abscesses, and less frequently abscesses in the brain and in other internal viscera. These abscesses are sometimes demonstrable by roentgenologic methods. They are identified as amebic in origin only by demonstrating the amebæ in the contents or in the walls.

Garland's résumé of the roentgenologic changes to be noted in the colon in cases of amebic colitis was very brief and not very specific. It indicated that he is not impressed with their importance. Druckmann and Schorr[4] and Golden and Ducharme[8] discussed these changes in greater detail in papers specifically devoted to that subject.

The diagnosis of amebic disease of the colon is made when *Endamœba histolytica* or one of its vegetative forms is demonstrated in the stools or in the tissues of the patient. The source of such organisms and vegetative forms is considered to be an intestinal ulcer or lesion. The well-known and widely quoted observations of Craig,[3] Faust[5] and Clark[2] have provided a good foundation in pathologic anatomy on which to construct a roentgenologic diagnosis of intestinal amebiasis. Their studies and those of others indicate: (1) that amebic lesions may be found in any part of the colon but with greatest frequency in the cecum; (2) that when such lesions are found in more aboral portions of the colon an older and possibly a more extensive cecal lesion is also present; (3) that ileal ulcers of amebic origin occur very rarely, and then usually in the severe, often fatal, cases and in patients with debility of high degree; (4) that amebic lesions have different sizes and shapes, varying from a size too small to be seen with the naked eye, to larger deep and crateriform undermined ulcers or shallow and spreading denudation of the mucous membrane; (5) that amebic ulcers are subject to secondary infection with bacterial organisms; and (6) that the segmental tumefactive granulomatous amebic lesions occur, often in the cecum, but also in the more aboral parts of the large intestine. Golden and Ducharme laid emphasis on the significance of the changes in the cecum, although 5 of their patients had changes elsewhere in the colon and rectum. Druckmann and Schorr recognized 2 types of amebic intestinal involvement: (1) a diffuse type, in which lesions are scattered all over the colon, producing roentgenologic changes which they said are similar to those of chronic ulcerative colitis; (2) a localized type, which is more characteristic and in which the colon is involved more or less segmentally in this order of frequency: cecum, ascending colon and sigmoid colon. The changes described in both papers are essentially the same, and except for

their peculiar distribution in the colon, they are the changes expected to be found in chronic segmental colitis of any etiologic type. The lumen of the portion of intestine involved is narrowed, its length is diminished, and the relief of the mucous membrane exhibits varying degrees of obliteration or distortion. These changes in the pattern of the relief of the mucous membrane signify ulceration; the deformity, narrowing and shortening signify inflammatory change, chiefly in the submucous structures. Evidently pathologico-anatomic change of relatively great magnitude must take place before amebic intestinal disease, or for that matter any kind of intestinal disease, will become manifest at roentgenologic examination of the colon. Pin-point ulcers and other lesions too small to be seen with the naked eye can hardly be expected to give convincing evidence of their existence, for roentgenologic evidence is essentially morphologic in character. Functional intestinal changes, such as local or general hypermotility, segmental irritability and tenderness over a particular intestinal segment may, I grant, be observed with such small lesions as their cause, but unless roentgenologic evidence of true morphologic change can be elicited at the same time, such phenomena must be striking and unequivocal if diagnostic value is to be given them. At the same time it is well known that minute lesions may occasionally give rise to alarming clinical symptoms and signs.

As Golden and Ducharme pointed out, deformity of the cecum without demonstrable evidence of change in the lowermost part of the ileum is the roentgenologic syndrome which makes the observer think specifically of intestinal amebiasis. The deformity may be marked and easily recognized, whether the contrast suspension is given by mouth, as Golden and Ducharme preferred to do, or by enema as Druckmann and Schorr considered preferable. The deformity may also be slight, amounting to little more than a barely perceptible but diffuse diminution in caliber of the cecal segment. Even minimal deformity will be accompanied by changes in the pattern of the relief of the mucous membrane. The changes are usually in the direction of obliteration, but distortion or more or less marked exaggeration of the relief may also be observed. In my opinion, changes of this low degree could not be elicited in a satisfactory manner with the use of the opaque meal alone. The determination of narrowing of an intestinal segment is essentially a test of its distensibility. This test requires active control of the intraluminal pressure. This is possible with the contrast enema, but not, it seems to me, with the contrast meal. Involvement extending to portions of the large intestine below the cecal level is easier to recognize at roentgenologic examination, simply because the deformity is larger and generally more pronounced. When amebic intestinal disease extends below the level of the hepatic flexure, it tends to have an irregular patchy type of distribution. Sites of preference for the lesions are the dependent portions of the colon. These are the sigmoid, colon and the rectum and the dependent portions of the hepatic and splenic flexures. The literature has references to diffuse and uniform involvement of long stretches of the large intestine, but I have not found descriptions of such involvement which were accompanied by illustrative roentgenograms, so I presume that it is rarely encountered. A confusing situation arises in patients who have other etiologic types of ulcerative colitis, and who are at the same time carriers of *Endamœba histolytica*. Such cases are probably best classified as instances of mixed infection.

I have described a secondary sign of amebic infection of the cecum which I still consider to be of value.[11] Golden and Ducharme seem to have misunderstood the description for they apparently tried to elicit the sign with the use of the contrast meal. The sign consists essentially in an unusually free flow of the contrast enema from the cecum, through

the ileocecal orifice, into the lower part of the ileum. It is almost always possible to make some of the contrast fluid enter and fill the terminal ileum after the cecum has been distended with the fluid, but often a special effort must be made to accomplish this especially in patients who have undergone appendectomy. When ileal filling is accomplished in this way, the ileocecal orifice is said to be patent. When the cecum has undergone even minimal morphologic change as a result of amebic infection, the ileocecal orifice often seems to be gaping rather than merely patent or capable of being forced open by sustained pressure, and the contrast fluid enters the ileum with remarkable ease and in unusual volume. To avoid the use of the word "patent" which had acquired a certain disrepute because clinical symptoms had been erroneously ascribed to so-called incompetency of the ileocecal valve, I used the term "patulous" to describe this condition of the structure. I have used this phenomenon consistently and with satisfaction not as diagnostic evidence of amebic intestinal disease, but as a signal to be on the alert for minimal roentgenologic evidence of morphologic change in the cecum when more obvious evidence was lacking. It is not always to be elicited, but in most of the cases of amebic typhlitis which I personally have observed it has been exhibited, and I have not seen it in connection with other etiologic types of ileocecal disease. I am unable to give an anatomic or physiologic explanation of the phenomenon. Golden and Ducharme observed it only rarely, but they used the contrast meal alone in the large majority of their roentgenologic examinations, although they recommend, as I do, the use of both the contrast meal and the contrast enema as the best roentgenologic approach to the diagnosis of intestinal amebiasis.

In the series of cases analyzed by Golden and Ducharme deformity of the cecum was observed more frequently in patients who had intestinal symptoms than in those who had no such clinical manifestations. How many of the carriers of *Endamœba histolytica* will be found to have roentgenologically demonstrable evidence of cecal or intestinal disease? Golden and Ducharme found such evidence in 25 of 58 patients who had the specific parasite in their stools. They concluded that the absence of cecal deformity is of no value in excluding intestinal amebiasis. Patients, however, are referred for roentgenologic examination of the intestine for many reasons and under various circumstances. Specific indications for microscopic examinations of the stools are not always at hand in intestinal amebiasis. Numerous examinations of the stools are often required to discover the organism. Positive roentgenologic findings not infrequently serve to stimulate a more vigorous and determined search for the organisms, or actually initiate the search. This is the real value of the roentgenologic examination of the colon in amebiasis. It may also be used with advantage to check the effects of anti-amebic therapy. Golden and Ducharme observed improvement in the roentgenologic appearance of the cecum in a few of the patients they were able to follow after treatment. In instances of marked cecal deformity, however, little change for the better was observed and they concluded that in later stages the changes produced are probably more or less irreversible. This observation was made also by others who had the opportunity of studying numbers of cases of intestinal amebiasis roentgenologically.

Neither Golden and Ducharme nor Druckmann and Schorr mentioned the contribution of the proctologic examination to the diagnosis of amebiasis. Buie and many others have frequently called attention to the typical morphologic characteristics of amebic ulceration as it appears at endoscopic examination of the rectum and sigmoid colon. The experienced proctologic observer apparently encounters no particular difficulty in making a distinction between amebic and

other etiologic types of proctosigmoiditis. I once made a survey of a series of carriers of *Endamœba histolytica* who came under observation consecutively over a period of several years. More than 50% of these patients had amebic ulcers in the rectosigmoidal region at proctoscopic examination. A slightly greater percentage of the patients had roentgenologic evidence of amebic intestinal disease. A large majority of the patients who had roentgenologic evidence of the disease also had rectosigmoidal amebic ulcers. Since that time I have held that the proctoscopic examination is almost as valuable an adjunct to the diagnosis of amebiasis as the roentgenologic examination is. This is a somewhat anomalous situation when one considers that the cecum is universally considered to be the site of earliest and probably severest amebic involvement. Apparently the clinical investigation for amebiasis should not be considered complete unless it includes scrupulous search for the organisms in the stools, roentgenologic examination of the colon after the contrast meal and the contrast enema, and proctologic examination, including, when indicated, the removal of a specimen of rectosigmoidal tissue for biopsy.

Gastro-intestinal symptoms of all grades of severity may be observed in hookworm disease, caused usually by *Necator americanus* or *Ancylostoma duodenale*. Hodes and Keefer,[9] in an excellent paper which may well be long remembered as a classic on this subject, confirmed the earlier observations of Krause and Crilly,[10] who described alterations in motility and pattern of the small intestine similar in almost all respects to those seen in the so-called deficiency states. Hodes and Keefer served in a general hospital of the Army of the United States in the Province of Assam in India where the incidence of hookworm disease is reputedly as high as in any place in the world. Their studies were conducted on military personnel who became infected in that area. The worms are infectious to man in their larval stage. The larvæ burrow through the skin, enter the veins and lymph vessels and are carried to the heart and lungs. Penetrating the capillary and alveolar walls they get into the bronchi, thence to the epiglottis and then are spilled into the esophagus and swallowed. In the intestine the larvæ develop into mature forms in about 1 month. By means of their mouth capsules they attach themselves to the intestinal mucous membrane whence they are nourished. Their preferred habitat is the duodenum, jejunum and upper part of the ileum. Itching is noticed in the burrowing stage by those infected. The term "ground itch" became a common one among soldiers in endemic areas. Cough, dubbed "foxhole cough" by troops, commonly appeared 1 or 2 weeks after the ground itch. Gastro-intestinal symptoms, appearing in from 6 to 25 weeks after exposure, began with nausea, vomiting, abdominal pain and diarrhea. Fever of low grade, abdominal tenderness, and leukocytosis were observed less frequently. Anemia was infrequent in the patients observed by Hodes and Keefer, but most of them were observed in the relatively early phases of the disease. One hundred and twenty-five American troops with proved ancylostomiasis were examined roentgenologically for gastro-intestinal changes; 60% of them proved to have significant alterations in the small intestine within 1 month after exposure to the parasites. The changes were described in intimate detail by Hodes and Keefer and the description is not easily paraphrased in brief form. Suffice it to say that the changes were analogous to, if not exactly like those seen in some of the cases of chronic avitaminosis and other deficiency states. Golden preferred to call them changes of "disordered motor function." They were characterized by exaggeration of the segmental and peristaltic contractions accompanied by distortion of the pattern of the mucous membrane. The abnormal intestinal picture appeared first in the upper part of the jejunum and spread from there to the lower part of the duodenum, to the rest of the jejunum and

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to the entire ileum. Hodes and Keefer discussed the various factors possibly operative in producing the changes. They concluded that the alterations could best be explained by postulating damage to the intramural nervous system of the small intestine. They regarded it as highly possible that the hooklike teeth of the parasites, embedded as they are deep in the mucous membrane of the intestine, could easily interfere with the network of nerve cells and fibers which extend to all portions of the intestine from Auerbach's subserosal, and Meissner's deep muscular plexuses. Because the abnormal appearance of the intestine persisted after the hookworms were destroyed by vermifugation, and because 40% of the patients with known hookworm disease failed to show evidence of intestinal abnormality, it seemed probable that the mere presence of the hookworms should be held responsible for the changes. In some cases the intestinal abnormality persisted for months after the patients were clinically well; in others, improvement was observed to occur early.

According to Garland, ascariasis is the most frequently encountered helminthic infection. The shadows of the worms have been demonstrated at roentgenologic examination of the intestine. They appear as elongated intraluminal shadows corresponding to the size and shape of the worms. Frequently the intestinal tract of the worm is outlined by the contrast material ingested by the worm from the host's intestine. Ascariasis is a diagnosis heretofore but rarely made at roentgenologic examination. It is probable that the incidence of this disease will not increase perceptibly in this country as a result of infestations acquired by our military personnel in foreign fields. Garland stated that the actual incidence of intestinal parasitism was low in naval personnel returning from the Pacific theater in the early years of the war.

Of special roentgenologic interests are the calcifications in the soft tissues of the body caused by parasites, some of which are strictly tropical in distribution, others observed in more temperate zones but much less frequently. The cerebral calcification occurring in certain cases of chronic malaria has already been mentioned. Diffuse cerebral calcification also may be discovered in cases of toxoplasmosis, caused by the sporozoan Toxoplasma, especially in case of the infantile type. The symptoms are those of encephalomyelitis. According to Garland, the calcified masses are usually bilateral, 1 to 3 mm. in diameter, and located in the cerebral hemispheres. Curvilinear streaks of calcification sometimes appear in the basal ganglia. Filariæ may die after having invaded the soft tissues, then they become calcified, to show up on roentgenograms as small linear or dotlike shadows, 1 to 4 mm. long, and 1 mm. wide, in the subcutaneous tissues, lymph nodes and scrotal lymphatics. Garland stated that they are difficult to recognize at times, but are fairly characteristic, having a distribution in the body different from calcified trichinæ, and much smaller than cysticerci. Cysticerci are the larval forms of *Tænia solium*, the pork tapeworm. They may invade any organ in the body. Calcification of the larvæ occurs but rarely before 5 years after infection. It is seen most frequently in the skeletal muscles, less often in the brain. In the muscles the calcified masses are elongated and lie in the direction of the muscle fibers. They vary greatly in size, shape, and number. In the brain the masses are smaller and tend to be scattered in the substance of the organ. Calcified trichinæ also appear in the skeletal muscles; they are small, compared with cysticerci, and do not tend to be elongated. Larger elongated, coiled and segmented shadows of calcification in the soft tissues of the leg and trunk have been described in dracontiasis, caused by the guinea worm, *Dracunculus medinensis*, a parasite formerly classified as *Filaria medinensis*. Garland stated that the large size of these calcified worms renders them readily recognizable, their favorite location being in the subcutaneous tissues of

the leg. Calcification in the submucous tissues of the large intestine and urinary tracts has been observed in schistosomiasis, caused by Schistosoma or blood flukes. The disease is also known as bilharziasis. The flukes enter the body through an abrasion of the skin. Garland stated that any organ of the body may be invaded, but the intestinal and urinary tracts are involved most frequently and most severely. Bleeding from one or both of these tracts is the most common symptom. The diagnosis is made by finding the ova in the excreta. The ova that have been deposited in the submucosal structures cause ulceration, necrosis, and ultimately calcification. This calcification appears on roentgenograms in streaklike form in the wall of the viscera affected or in ovoid masses which have the appearance of calculi or calcified papillomas.

The principal tropical disease manifested by roentgenologically demonstrable lesions of the lungs are: (1) distomiasis, caused by the trematode *Paragonimus westermani;* (2) echinococcosis, caused by the larval form of the dog tapeworm; (3) Q fever, caused by *Rickettsia diaporica* or *burneti.* Garland made brief descriptions of the roentgenologic changes produced in the lungs in all of these diseases. None of them are very characteristic, except the hydatid cysts of echinococcal origin.

Lesions of bones are seen in a number of tropical diseases. In echinococcosis irregular cystic lesions develop, especially in the ribs and pelvic bones, which resemble fibrocystic disease of bone or osteolytic metastatic carcinoma. Osseous lesions, similar to those seen in syphilis, develop in the diseases caused by various forms of Treponema. Yaws, pinta, and bejel are examples. Goundou, a chronic sclerosing osteoperiostitis of the superior maxillæ, and gangosa, a destructive ulcerating disease of the nose and palate, are tertiary forms of yaws, caused by *Treponema pertenue.* The diseases respond favorably to antisyphilitic treatment. Lesions of bone, more or less typical, are also encountered in leprosy, Madura foot, ainhum and in various types of tropical ulcers caused by spirochetal and bacillary organisms.

Roentgen therapy has been used with favorable results in the treatment of the lymphangitis, lymphadenitis and chyluria of filariasis. It also was used successfully in the treatment of acne vulgaris and epidermophytosis, diseases which are not tropical, but which tend to undergo severe exacerbation under some tropical conditions.

Very briefly stated, these are the principal considerations that seem thus far to have developed from recently gained experience with roentgenologic manifestations of tropical diseases. Instances will probably be infrequent when the roentgenologic findings will yield the final diagnosis. Roentgenologists and their consultants in internal medicine should be prepared, however, to think in terms of tropical diseases and their late sequelæ. Physicians who have had experience in tropical areas with our armed forces probably have become accustomed to keeping these possibilities in mind. Those who have not had such opportunities will add this group of diseases to those with which they have, until now, been more familiar.

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PHYSIOLOGY

PROCEEDINGS OF

THE PHYSIOLOGICAL SOCIETY OF PHILADELPHIA

SESSION OF MARCH 19, 1946

OWING to the meetings of the Federation of American Societies for Experimental Biology at Atlantic City in March, the March session of the Physiological Society of Philadelphia was not held.

BOOK REVIEWS AND NOTICES

RECENT ADVANCES IN NEUROLOGY AND NEUROPSYCHIATRY. By W. RUSSELL BRAIN and E. B. STRAUSS. Fifth ed. Pp. 363; 32 illus. Philadelphia: Blakiston, 1945. Price, $5.00.

THE latest edition of this "ancillary text-book of neuropsychiatry" is a welcome revision of a book whose usefulness is demonstrated by the fact that this is a 5th edition. It is written with a clarity apparent in other works by the same author. It is particularly useful since it reviews literature not always generally available during the war years.

Of particular interest are the studies of conditions seen in connection with war wounds, especially injuries of the brain and of the peripheral nerves; recent studies of vestibular functions and Ménière's syndrome, especially in this connection the re-interpretation of caloric vestibular tests. An interesting section discusses the demyelinating diseases of the nervous system, and a summary of neurotropic virus diseases also appears here. In keeping with its extended use is a section on electroencephalography.

The general contents are more concerned with the neurologic aspects of neuropsychiatry than the psychiatric. This point of view is illustrated in the sentence discussing the difference between Pick's disease and Alzheimer's disease, "to put it pithily, one might say that if one performed prefrontal leucotomy on a case of Alzheimer's disease one would produce the clinical picture of Pick's disease."

The book can be recommended highly.

G. G.

SYNOPSIS OF GYNECOLOGY. By H. S. CROSSEN, M.D., F.A.C.S., and R. J. CROSSEN, M.D., F.A.C.S. St. Louis: Mosby, 1946. Price, $3.00.

THIS 3rd edition of Crossens' "Synopsis of Gynecology" is like its predecessors, intended principally for "medical students who intend to follow distant branches of their profession" and for practicing physicians as a "compact presentation of the outstanding features" of gynecology.

The publishers have compressed a good short text-book into 233 pages by clever use of space and by marked reduction in the size of the print. The latter feature, however, renders prolonged reading rather difficult. The chapters, with their subdivisions, are clearly titled and the index is accurate and ample.

The subject matter is presented in a concise and readable manner and very few deletions are made for the sake of brevity. The book contains 135 accurate, clear illustrations, many of which are diagrammatic. The chapters upon genital anatomy and physiology and upon menstrual disturbances are up-to-date and clearly written. The section upon genital embryology and malformations is exceptionally lucid and practical. The discussion of ovarian tumors is inadequate, however, as the solid tumors of the ovary receive only scant mention.

As is true of most text-books upon a living subject, the lapse of time between the editing and the publishing has permitted the appearance of new subject material that could not be included. The book contains a minimum of information concerning chemotherapy in

gynecology and no information concerning the antibiotic agents or the Rh blood factor.

It is doubtful that the "Synopsis" will be acceptable as a text-book for medical students whose ideas have not been crystallized concerning specialization at such an early age. On the other hand, it is admirably adapted for the purpose of a rapid review or a hurried reference by students, house officers, and busy practicing physicians. F. P.

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